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(71) Applicant *(for all designated States except US)*: WALLAC OY [FI/FI]; P.O. Box 10, FIN–20101 Turku (FI).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LEHTO, Ari [FI/FI]; Raekuja 3, FIN–00700 Helsinki (FI). KOJOLA, Hannu [FI/FI]; Turuntie 346, FIN–21870 Riihikoski (FI). LÖVGREN, Timo [FI/FI]; Yliopistonkatu 2 H 155, FIN–20100 Turku (FI). LÖNNBERG, Harri [FI/FI]; Vatjankatu 3, FIN–20750 Turku (FI).

(74) Agent: TURUN PATENTTITOIMISTO OY; P.O. Box 99, FIN–20521 Turku (FI).

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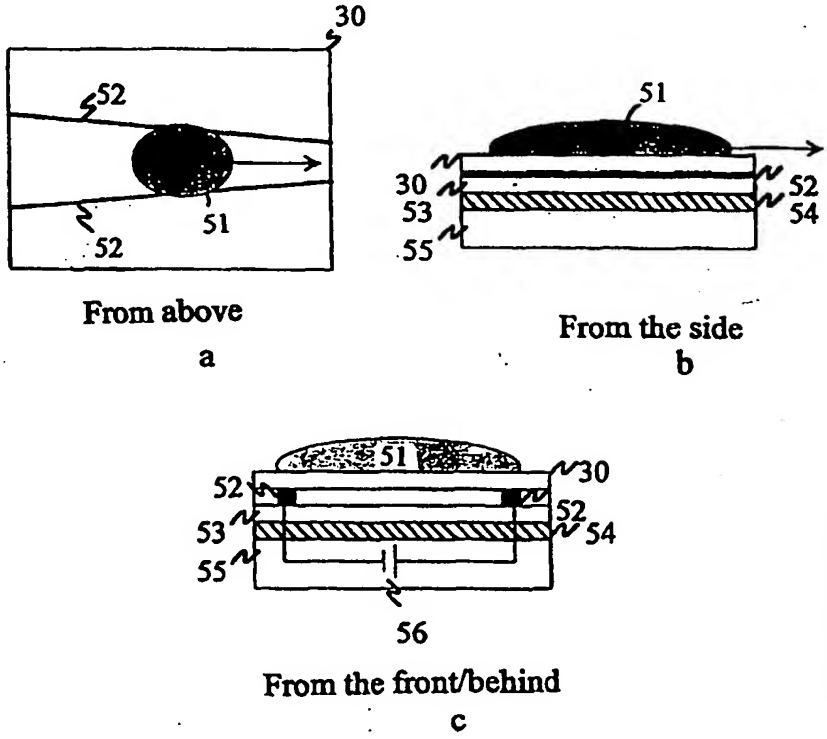
(57) Abstract

The invention relates to a method for carrying out a chemical analysis, in particular a bioaffinity assay on small liquid volumes, in which method droplets (51) are formed from a sample solution, and from one or more solutions used in the assay, onto a surface (30) repellent to the droplets, on which surface the droplets (51) are transported by using an electric field of changing strength in order to fuse the droplets (51) in the desired order and optionally to carry out an analysis, to shake the droplet (51), to regulate the temperature, and/or to wash the solid carrier optionally contained in the droplet (51). The invention also relates to a device for use in carrying out this method, the device having means (52) for transporting and fusing liquid doses, and optionally for dosing a liquid volume which contains the sample and at least one liquid volume which contains a reagent and, when a solid carrier is used, a washing liquid volume, for shaking the reaction mixture, for regulating the temperature and/or for analyzing the reaction product, as well as a liquid–repellent surface (30) and electrodes (52) fitted as transport tracks in the vicinity of the surface (30), and means for transferring liquid volumes as droplets onto the surface in the area of the said transport tracks.



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Method and device for carrying out a chemical analysis in small amounts of liquid

The invention relates to a method and device for performing a chemical analysis on small liquid volumes, the device having members for dosing a liquid volume containing the sample and at least one liquid volume containing a reagent and, if a solid carrier is used, a volume of washing liquid; for transporting and fusing the liquid doses; for shaking the reaction mixture; for changing the temperature of the reaction mixture; for regulating the temperature of the reaction mixture; and/or for analyzing the reaction product, in particular for performing a bioaffinity assay.

Time-resolved fluorescence and lanthanide chelates were mentioned in the literature for the first time in 1978 [1]. The usability of the technology for immunoassays was proved over ten years ago [2]. The high specific activity of the label made possible highly sensitive non-competitive immunoassays, since the nonspecific binding of labelled reagents was minimal [2,3]. Subsequently, a number of label options with high specific activities have been implemented. At the same time, hybridization assays of nucleic acids have increased rapidly, in particular owing to effective replication methods, such as PCR [4,5]. It is evident that, in order to accomplish optimal sensitivity in analytical applications, such as immunoassay or nucleic acid hybridization, there is required a label which has a high specific activity and consequently a low nonspecific binding of the labelled component.

The miniaturization of the assay format further emphasizes the importance of improved detection technologies [6]. The develop-

ment of miniaturized assay formats has been proposed for the performing of immunoassays [6], for nucleic acid hybridization for the detection of mutations, for the measuring of protein production [7 - 9] and for genome sequencing [10,11], as well as for the use of combinatorial peptide chemistry for the investigation of biomolecular interaction [12,13]. By this is in general meant locally separate reactive small areas on the substrate surface. Thus a large number of different biomolecules have to be synthesized in them, while retaining the potential biologic activity [13]. Alternatively, separately identifiable microparticles can be used for these applications. There is more available surface area in a microparticle than in a spot having the same diameter. Likewise, productional viewpoints also favor the use of microparticles instead of small sets of areas to be immobilized.

Research and development of miniaturized assay formats has been based on the use of conventional fluorochromiums as labels. The use of autofluorescent lanthanide chelates is advantageous for the assay format owing to the low background fluorescence, the high specific activity of the labels and their low nonspecific binding. The technology requires an effective, high-resolution detection system for measuring the binding reproducibly and quantitatively from miniaturized complex matrices. Up to the present, preliminary assay material has been produced by means of fluorometry or a confocal fluorescence microscope, by using laser as the excitation source and fluorescein or phycoerythrin as the label [6,9,12].

For the automation of assay processes, conventionally systems have been constructed for the performance of the reaction steps and the required manipulation of the liquid on a microtiter plate or in open vessels of the corresponding size category.

However, these systems are large in size and produce a large amount of solid and liquid waste.

For purposes of miniaturization, a large number of different microfluid options have been constructed, most commonly with the help of channels etched on silicon or glass [14, 15]. The disadvantages of these channel system options include the formation of pockets and sharp angles owing to the etching lines following the crystal planes. Consequently, washing becomes difficult and the success of the assay becomes uncertain. Likewise, there are encountered risks of a pocket difficult to wash, formed between the cover plate required by the channels and the frame joint. Furthermore, linking the channel system to parts outside the device requires pipe joints, which break easily and contain pockets difficult to wash.

The conventional micromechanical options are not suitable for the automation of an assay using microparticles as solid carriers, since structures implemented by using silicon-structured valves or membranes are not capable of handling particle suspensions. If biomolecules are immobilized on a substrate, the microfluid part will be disposable, in which case the costs and the amount of solid waste will increase. Likewise, there are encountered the above-mentioned productional yield problems if many different biomolecules are fixed to the same substrate.

The combining of different assay protocols in one and the same device is difficult unless the automation system allows freedom in the arrangement of protocols. For this reason, competitive and non-competitive immunoassays, nucleic acid hybridizations, etc., are difficult to combine in a system in which selective immobilizations are made on the same substrate.

Washizu [16] investigated the properties of a sequential droplet transport track and proved that it can be used for transporting small liquid droplets. Pethig [17] investigated a corresponding system for the transport of cells and microspheres. The use of an electric field gradient for the moving of the movable targets was not disclosed in either one of the publications. Hozumi and Takai [18] showed that highly hydrophobic surfaces can be made by the PECVD method. The researchers observed that the contact angle of a liquid droplet is affected additionally by the roughness of the surface so that a suitably rough surface is better than a smooth one.

It is an object of the present invention to provide a method and device for the automation of the chemical analysis of small liquid volumes, for example in clinical analysis, such as immunoassays, nucleic acid assays, combinatorial chemistry, receptor assays or enzymatic assays, etc. This has been achieved as is stated in the accompanying patent claims.

The device and method according to the invention are particularly suited for bioaffinity assays, preferably when microparticles are used as solid carriers, but for a person skilled in the art it is evident that they are also generally applicable to other chemical analyses in which the assays are carried out by using small liquid volumes, either by repeating the same assay in time or by carrying out several assays successively or in parallel.

The advantages achieved by means of the invention include the fact that it is possible to use small liquid volumes, in which case also the reagent requirement is small and possibly also at least a portion of the reagent is advantageously fixed to a solid carrier. The requirement of an expensive label material

is also low. The method and device according to the invention are easy to automate, and they provide high precision, speed and capacity owing to the speed and the ease of providing process elements in parallel. In addition, the device according to the invention can be easily manufactured by planar technology.

The invention is elucidated below in greater detail with the help of figures, wherein

Figure 1 depicts the principle of the course of an analysis according to the invention;

Figure 2 depicts the forming of an immunocomplex on the surface of a microsphere;

Figure 3 depicts the detaching of a droplet from a capillary tip by using an energized tip;

Figure 4 depicts the detaching of a droplet from a capillary tip by using mechanical vibration;

Figure 5 depicts the transport of a droplet in a gradient field;

Figure 6 depicts the limiting of a droplet on the surface to a small space;

Figure 7 depicts one optional electrode configuration;

Figure 8 depicts another optional electrode configuration;

Figure 9 depicts the transport of a droplet on a sequential track and the structure of the track;

Figure 10 depicts reciprocal moving of a droplet by means of gradient electrodes;

Figure 11 depicts the changing of the travel direction of a droplet on a gradient track;

Figure 12 depicts the electric charging of a droplet by the use of a field emitter tip;

Figure 13 depicts the transfer of charges into a droplet by mediation of gas molecules;

Figure 14 depicts the increasing of the mobility of droplets by the vibration of the track network;

Figure 15 depicts the fusing of droplets on a gradient track;

Figure 16 depicts the transport and fusing of droplets on a sequential track;

Figure 17 depicts gradient electrodes coupled in series;

Figure 18 depicts the steps of the washing of a sphere with the help of gradient electrodes;

Figure 19 depicts the washing of a sphere by means of a sequential structure;

Figure 20; depicts the washing of a droplet by its being sucked underneath the substrate material;

Figure 21 depicts the heating of a droplet by means of resistive electrodes;

Figure 22 depicts the local heating of a droplet in an RF field;

Figure 23 depicts certain options for implementing cyclic temperature control;

Figure 24 depicts the performing of an optical measurement on a droplet;

Figure 25 depicts a substrate structure based on intersecting tracks of travel;

Figure 26 depicts a substrate structure based on non-intersecting tracks of travel, most of the liquids being dosed onto the track from above;

Figure 27 depicts a substrate option based on two separate surfaces;

Figure 28 depicts the principle A of an immunoassay taking place on a microparticle surface and the principle B of a hybridization assay;

Figure 29 depicts a calibration curve (•) and a precision profile (■) for a PSA assay in a volume of

1.67 μL per microparticle. The dotted line indicates the assay sensitivity 5 ng/L;

Figure 30 depicts the hybridization of a non-mutation-specific synthetic target oligonucleotide for the assay of cystic fibrosis F508 mutation;

Figure 31 depicts a typical calibration curve obtained in a homogeneous energy transfer assay for a β_{hCG} assay;

Figure 32 depicts a typical calibration curve obtained in a homogeneous energy transfer assay for a PSA assay.

The principle of the course of an analysis according to the present invention is shown in Figure 1. In the method, the sample 11 and the reagents participating in the reaction, in Figure 1 the carrier 12 and the label 13, are turned into small liquid droplets, which are transported to the incubation section 14. Preferably there is used in the assay a solid carrier, such as microparticles serving as solid carriers in a bioaffinity reaction. Capturing molecules to which the sample molecules bind selectively have been immobilized on the solid carriers. The labels 13, which are preferably fluorescent labels, are also transported to the incubation section 14, where they attach selectively to the sample molecules. Each component fed in may have its own separate route from the feeding point to the incubation section 14, or they may use the same routes at least in part.

In the incubation section 14 the liquid droplets are fused together, and there form structures, for example according to Figure 2, in which the sample (analyte) 23 binds to the antibody 22 in the solid carrier 21, the labelled bioaffinity com-

ponent 24 becoming attached to the sample. If the label used is, for example, fluorescent, by exciting the label molecule by means of suitable light it is possible to detect, on the basis of the fluorescent light 25 emitted by the component, the quantity of labelled bioaffinity component 24 bound to the sample.

After mixing in the incubation section 14, the droplet is transferred in accordance with Figure 1 to washing 15. The purpose of the washing 15 is to remove any excess unbound label from the assay space. After the washing 15 the droplet is transferred to the detection zone for the detection 16 of the label bound to the sample 23, for example by means of a fluorescence detector.

Figure 3 shows one method for the dosing of a liquid 32 as a droplet 31 onto the surface 30 by means of a capillary 33. At the tip of the capillary 33 there is a conductive portion 34, to which voltage is applied in order to give the liquid 32 emerging from the capillary, for example a negative charge. By giving the liquid droplet 31 a charge of the sign opposite to that of the closest electrode 35 on the transport track, the droplet can be subjected to a greater attraction than without the charging of the liquid.

In Figure 4 the tip portion of the capillary 33 is equipped with an actuator 40, which produces a mechanical vibration, facilitating the detaching of a droplet 31, at the tip of the capillary 33. The actuator 40 may be, for example, a piezoelectric vibrator.

Figures 5a, 5b and 5c show an example of the operation of a transport track based on an electric field gradient, used for the transport of a sphere located inside a droplet, a sample

droplet or a label molecule droplet, as seen from above the transport track (Figure 5a), as a cross-sectional representation from the side relative to the droplet travel direction (Figure 5b) and as a cross-sectional representation direct from the front or behind relative to the droplet travel direction (Figure 5c).

The transport track shown in Figures 5a, 5b and 5c comprises a preferably hydrophobic, non-conductive surface 30 which is in contact with the droplet 51 being transported and, located under the surface 30, an electrode pair 52. The electrode pair is located between the surface 30 and an insulation material layer 53. The insulation material layer 53 is on top of a ground plate 54. The electrical couplings of the electrodes 52 are taken through the insulation material layer 52 and the ground layer 54 to the substrate material 55 or, when so desired, to a completely separate electric circuit 56 in order to couple voltage to the electrodes 52.

The substrate material 55 may be silicon, quartz or some other material the properties of which are optimal in terms of the analysis method used, for example when fluorescence measurement is used the fluorescence of the substrate material is minimal. Preferably a planar substrate is used, but substrates of other kinds can also be used.

In order to ensure that the droplets will remain on the track, the surface can be made three-dimensional, for example by scratching or etching grooves corresponding to the shape of the track structure into the surface. Furthermore, surface forms corresponding to the track structures can be prepared by, for example, the SOI (Silicon On Insulator) technique, known per se, in which a single-crystal silicon-silica sandwich structure

is prepared on top of a silicon disc by attaching a pure silicon disc to an oxidized silicon disc and by thinning the pure disc by grinding to the desired thickness [22]. By the SOI technique, structures corresponding to the track structure can be made easily by providing a pattern in the top layer of silicon and by etching the silica from in between. Instead of silica it is also possible to use some other insulating material.

Furthermore, it is possible to prepare in the track structure spots in which a droplet can be enclosed in a limited space, for example, for the duration of heating. Thereby, for example, evaporation losses occurring during heating can be reduced. Figure 6 shows the enclosing of a droplet 51 travelling on the surface 30 under a casing 61 lowered onto the track. The casing 61 is preferably elastic at its edges in order to provide a tight joint between the surface 30 and the casing 61.

When so desired, the electrodes 52 can be placed in a manner other than that shown in Figures 5a, 5b and 5c. The electrodes 52 may be located, for example, on the sides of the droplet to be transported, as shown in Figure 7, in which case the droplet can be subjected to a stronger electric field than in a case in which the electrodes 52 are located in a different plane relative to the droplet 51, as in Figure 5c. At the same time, a three-dimensional structure, which promotes the remaining of the droplet on the transport track, is achieved.

Figure 8 depicts a third example of the placement of the electrodes 52. In Figure 8 the electrodes 52 are located above the droplet 51 being transported. An option such as this makes possible the separation of the surface 30 coming into contact with the droplet 51 and the electric parts from each other. In this case the surface 30 coming into contact with the droplet

51 can be implemented easily, for example by using a disposable, easily replaceable discrete or continuous plastic tape, in which case the surface 30 can be replaced by another without the necessity of also replacing the electric parts; this makes it possible, for example, to replace the surface at desired intervals or always when the sample batch is changed.

The operation of the gradient electrodes is based on the phenomenon, known from the classical theory of electricity, that a moving insulator tends to fill the space between capacitor plates coupled to voltages of opposite signs. This is due to the fact that the electrical potential energy of the insulator is minimized when the insulator fills the capacitor. If the electrodes are not parallel (Figure 5a), the electric field between the electrodes is not homogeneous; the electric field is stronger the closer the electrodes are to each other. The electric field between the electrodes has in this case a gradient, from which there comes the name "gradient track" for the transfer track using electrodes of the type described. When a droplet 51 of a dielectric material arrives in the electric field of electrodes according to Figure 5a, it tends to minimize its electric potential energy by moving towards the higher field strength, i.e. in Figure 5a in the direction according to the arrow, where the distance between the electrodes is smaller.

Alternatively it is possible to use for the transport of droplets a sequential electrode structure in accordance with Figures 9a and 9b. When, by means of an electric circuit 81, voltage is applied to one of the electrodes 52, an electric field is formed, and the droplet 51 tends to settle so that its electric potential energy is minimized. This is achieved in a situation in which the droplet 51 covers as large a proportion as

possible of the lines of force of the electric field, i.e. when the droplet 51 is located on top of the electrode which generates the electric field. By coupling of the electric field in turn to the following electrode 52, the droplet 51 can be made to move in the desired direction. The reciprocal moving of the droplet 51 provides, with the help of the sequences, the mixing of the droplet by shaking.

The shaking of the droplet on a gradient track can be carried out by using an electrode configuration according to Figure 10a, made up of two electrode pairs 92 and 93 coupled crosswise. When the first electrode pair 93 is energized, the droplet 51 moves in accordance with Figure 10b to the right. When the voltage is coupled to the other electrode pair 93, the direction of travel of the droplet 51 will be, in accordance with Figure 10c, to the left. By the shifting of the voltage in turn to the first and to the second electrode pair, the droplet 51 is brought into a reciprocal movement in order to mix the droplet by shaking.

By using the electrode configuration according to Figure 10a it is also possible to implement the intersection areas of the gradient track, if each electrode part is controlled independently. Figures 11a and 11b show how the travel direction of a droplet 51 coming from the left can be turned by 90 degrees. Of course, also direction changes other than those depicted in Figures 11a and 11b can be implemented in a corresponding manner, but in order to simplify the drawing, only some of the electrodes are depicted in the figure.

In Figure 11a, voltage is applied to the first 94 and the second 96 electrode parts, in which case the droplet 51 travels in Figure 11a to the right. After the droplet has reached the

end of the first 94 and the second 96 electrode parts, voltage is applied to the third 95 and the fourth 98 electrode parts, whereupon they together form a new electrode pair, the electric field formed between them generating, in accordance with what has been described above, in the droplet 51 a force under the influence of which the droplet is directed in accordance with Figure 11b downwards.

The charge of a droplet can be used for influencing the movement of the droplet on a sequential track. Figures 12 and 13 show two examples of methods by which charge can be transferred to a droplet. In Figure 12, charge is transferred to the droplet 51 by means of a field emitter tip 91 made in the substrate. When so desired, a plurality of tips 91 can be prepared, for example as a row or a matrix, on the route of the droplet 51. A field emitter tip 91 is typically in contact with the droplet 51, but it is also possible to make on top of the emission tip 91 a very thin insulation layer, typically of the thickness of a few atom layers, in order to avoid non-desirable reactions. When, for example, a negative voltage is applied to the field emitter tip 91, electrons transfer to the droplet 51 and it obtains a negative net charge. If the droplet 51 and the electrode under it have a charge of the same sign, they repel each other. With a positive track electrode it is now possible to pull the droplet and with a negative one to push it.

Charges can also be transferred to the droplet 51 via the air by using a sharp tip 101 in accordance with Figure 13. From the tip 101 the charge transfers first to gas molecules in the air and from them to the droplet. The phenomenon is known by the name "electric wind." The tip 101 may also be at so low a level that it comes into contact with the droplet 51 as it passes the

tip 101, whereby the charge is transferred directly to the droplet 51.

The movement of the droplet can further be affected by mechanical vibration. Figure 14 shows one such mechanism. On top of the substrate 55 there is mounted a vibration means 111, for example a piezoelectric element, on top of which there is placed the surface 30 in contact with the droplet 51, and possibly at least some of the other parts required by the track structure (not shown in Figure 14). The surface 30 can be moved rapidly in vertical or horizontal directions, or as a combination of these movements, by using the vibration means 111. Under the effect of inertial forces the droplet 51 remains in place, and the surface 30 moves relative to it, tending to detach the droplet.

Figure 15 shows the fusing of droplets on a gradient track. On the surface 30 there has been created a track wherein the first route, formed by a first electrode pair 121, and the second route, formed by a second electrode pair 122, meet within the fusion zone 126, from which they continue as a third route, formed by a third electrode pair 123. The lower electrode of the first electrode pair 121 and the upper electrode of the second electrode pair 122 have the same potential. The purpose of the control 127 is to improve the directing of the droplets arriving along the first route and the second route to the third route, formed by the third electrode pair 123.

In Figure 15, the second droplet 125, which has arrived along the second route, is waiting in the fusion zone 126 for the first droplet 124, arriving along the first route. When the first droplet 124 arrives in the fusion zone 126, the droplets

touch each other and join into one droplet. Simultaneous arrival also fuses the droplets.

Figure 16 shows a corresponding fusing of droplets on a sequential track. On the surface 30 there has been created a track in which a first route 131 and a second route 132 meet in the fusion zone 126, from which they continue as a third route 133. In Figure 16, the first droplet 134, arriving in the fusion zone 126 along the first route 131, contains a solid carrier 135. In the fusion zone 126 the first droplet 134 touches the second droplet 136, arriving along the second route 132, whereupon the droplets fuse, forming a third droplet 136, which contains the solid carrier 135 and which is transported along the third route 133 away from the fusion zone 126.

Gradient electrodes can be coupled in series in accordance with Figure 17. When the droplet 51 reaches the end of the first electrode pair 141, the voltage (Vs) of the first electrode pair 141 is zeroed, and respectively voltage (Vu) is applied to the second electrode pair 142, whereupon the droplet 51 moves to the second electrode pair 142. If the kinetic energy of the droplet 51 is sufficiently high, it can be caused to travel from one electrode pair to another even without the sequencing of the voltage.

By the coupling of gradient electrodes in series in accordance with Figure 17, a simpler electric circuitry is achieved than with sequential electrodes, since two electrode pairs at a time can be sequenced.

If an analysis method which requires separation is used, it is preferable to wash the droplet before detection. The purpose of the washing stage is to remove from a droplet which contains a

solid carrier all unbound label substance. Figures 18a and 18b depict one option for carrying out the washing by means of gradient electrodes. First the droplet is directed into a narrow electrode gap formed of three electrode pairs 152, 153 and 154, in such a manner that the droplet 151 stretches into an oblong shape, as shown in Figure 18a. The electrodes 152, 153 and 154 are dimensioned so that, when solid carriers are used, the solid carrier is always left on the side of the first electrode pair 152. After the stretching of the droplet 151, voltage is removed from the middle electrode pair 153, whereupon the droplet 151 is divided, in accordance with Figure 18b, into a portion 156 which contains the solid carrier, if any, and which remains at the first electrode pair and a portion 155 which remains at the third electrode part and which is to be removed. Thereafter the portion 155 to be removed is transported away, and fresh washing liquid is introduced into the washing section, and the sequence is repeated for the necessary number of times.

Figures 19a and 19b depict a washing station of a corresponding type, implemented by using sequential electrodes. A droplet which contains a solid carrier 165 is brought along the first sequential track 160 to the washing zone, where the track runs between a first 162 and a second 163 limiting member. The limiting members 162 and 163 are designed so that they narrow the track. When a solid carrier is used, it is advantageous to design the limiting members 162 and 163 so that at a certain point of the washing zone the limiting members 162 and 163 are so close to each other that the solid carrier 165 cannot travel forward between them. Thereupon the droplet 164 stretches into an oblong shape without the solid carrier coming to the tip portion 169 of the elongated droplet.

It is preferable to dimension the limiting members 162 and 163 in such a manner that at least one of the limiting members, in Figure 19a the second limiting member 163, does not extend across the intersecting second transport track 161. In this case the tip portion 169 of the elongated droplet in Figure 19a can be detached as a separate droplet 166 according to Figure 19b by sequencing the electrodes of the second track 161. Thereafter the portion 166 to be removed is transported away, and a new washing liquid droplet 167 is introduced into the washing zone, and the cycle is repeated the necessary number of times.

Figure 20 depicts an example of how a droplet 51 can be sucked by capillary forces through a small hole 171 or small holes into a suction medium, for example a felt 172, under the surface 30. New washing liquid droplets can be introduced according to need. In order to align a droplet to be washed with the holes and to limit the movements of the droplet it is possible to place around the holes, for example, pins manufactured using the SOI technology to limit the movement of the droplet.

It is possible to provide on the track one or more points at which the surface temperature can be regulated with precision and speed, for example, in order to produce temperature cycles necessary for PCR replication or for the incubation of the target. It is preferable to heat the replication point by using, for example, resistive transfer elements 181, as in Figure 21, or separate heating resistors and measuring elements made under the surface. At the heating point the substrate can be thinned and/or its surroundings can be etched open where necessary, so that the thermal contact with the heating-area surroundings, which are to be maintained cold, is optimal in terms

of an effective budget, the heat mass and sufficiently rapid cooling.

On the other hand, the heating can be targeted directly at the droplet by constructing the droplet as part of a microwave circuit, the transfer line or the resonator, or by subjecting the droplet to a high-frequency (RF) electric field. Figures 22a and 22b show two alternative electrode configurations for this purpose. In the configuration of Figure 22a, two transport-track electrodes 191 on the substrate are used as high-frequency electrodes, and in Figure 22b an extra capacitance electrode 192 has been placed above the track. High-frequency voltage is coupled between the capacitance electrode and the substrate, or between the capacitance electrode 192 and the track electrode 193. For a person skilled in the art it is clear that the position of the electrodes producing the RF field relative to the droplet to be heated can be varied.

It is typical of another embodiment of the implementation of thermocycling that the transport groove has in succession zones of different temperatures over which the droplet carrying the reaction mixture to be thermocycled is transferred. The speed of the droplet is preferably chosen so that the droplet has time to be at each point until the temperature has leveled sufficiently for the relevant stage of replication. This embodiment has the advantage that the substrate does not have temperatures to be changed dynamically, in which case the moving thermal mass is made up of only the reaction mixture itself. Furthermore, the progress of the assay process may be, in accordance with Figure 23a, synchronically progressing, in which case each transport track section 201, 202, 203, 204 has its own temperature, at least some of them being, when so desired, the same temperature, and there may be at each point,

when so desired, a sample droplet or a sample and washing substance in alternation, as needed.

Figure 23b shows the implementation of cyclic heating by using a structure in which the droplet to be heated is moved reciprocally between two track zones 205 and 206 having different temperatures. Thereby a more compact structure is achieved than in the option of Figure 23a, but the capacity is lower, since the next droplet can be brought to the heating zone only at the end stage of the previous cycle, whereas in the option according to Figure 23a there may simultaneously be several droplets at different stages of the heating cycle at the different points of the heating zone. In the option according to Figure 23b, the temperatures T1 and T2 need not be constant; when so desired, they can be regulated as a function of time. Furthermore, there may be on the transport track several spots at different temperatures which can, when so desired, be prepared as a cycle section according to Figure 23c, which in Figure 23c contains three stages 207, 208 and 209.

Furthermore, it is, of course, possible to make combinations of the structures described above, according to the assay protocol and capacity requirement at each given time.

The quantity of sample molecules bound to the surface of the solid carrier can be determined, for example, by detection based on fluorescence. Time-resolved fluorescence is an advantageous method for the detection of biocomplexes, since the classification of solid carriers, implemented by using different labels, can be combined with it in parallel.

The molecules on the surface of the solid carrier are identified by using fluorescence measurement, which can be

performed according to the principle depicted in Figure 24. A droplet 210, inside which there is a solid carrier 211, such as a polymer sphere, is driven under an optical fiber 212. The excitation light 214 is introduced by using the optical fiber 212 to near the sample droplet 210 and is focussed by means of a microlens 213, formed by the fiber end, on the solid carrier 211. Respectively, the fluorescence light 215 emitted by the sample substance bound to the solid carrier 211 is collected into the fiber 212 by means of the microlens 213 at the end of the fiber, and is transported in the fiber 212 for analysis. For a person skilled in the art it is clear that the detection can also be implemented by using lens optics or an optical system constructed from mirrors.

In terms of the detection it is preferable to know the location of the solid particle as precisely as possible. The location of the solid particle can be estimated with precision in advance by using so narrow an electrode pair that the space left between them is smaller than the diameter of the solid carrier. In this case the solid carrier is left between the electrodes, as shown in Figures 17a and 17b, discussed in connection with the washing. When the diameter of the solid carrier and the distance between the electrode pair are known, the location of the solid carrier, and thus also the most preferable spot for detection, can be defined. At its simplest the detection can be performed at the washing point, in which case a separate electrode pair is not needed for the detection point.

It is essential for the operation of the transport track that the surface of the channel system remains hydrophobic. For this purpose the surface can be coated with a thin hydrophobic membrane, for example "Teflon", AKD (alkylkerene dimer), such as the product marketed by Raisio France under the trade name

Raisares A, a lipid membrane, or the like. In the present invention, preferably PECVD (= Plasma Enhanced Chemical Vapour Deposition) polymer [21] is used as the topmost layer. The surface can be roughened in a controlled manner by using preferably porous silicon instead of producing the roughness by changing the PECVD parameters. The roughness of the surface of the transport tracks is preferably within the range 0.1-10 μm.

The control of the droplet movement tracks by means of an electric field and/or mechanical controls can be further improved by making the surface in the area of the droplet-transporting transport tracks preferably less hydrophobic than outside the transport tracks. Likewise, it is advantageous to dimension the surface roughness so that the surface is less rough in the area of the transport tracks than in the other parts of the surface.

It is preferable to encase the device according to the invention. The purpose of the encasing is to produce inside the device a microclimate suitable for the analyzer. The most important properties of the microclimate include the correct temperature and moisture. The casing must also prevent the access of dust and other impurities to the inside of the device. The purpose of moisture control is to create an equilibrium between the water evaporating from the droplet and the water condensing into it. Temperature control, for its part, is necessary for creating the correct reaction conditions for the chemistry. The casing preferably also has sites for all of the liquids required.

The architecture of the device according to the invention can be implemented in a number of different ways. In a two-dimensional architecture the system can be implemented both by using sequential electrodes as a track (Figure 25a) in which

the travel direction of the moving droplets can, when so desired, be changed, and by using gradient electrodes as a track which progresses in one direction but branches out (Figure 25b). In addition to its compact structure the option has the advantage that the liquid connections can be placed on the sides of the transport plane, which simplifies the structure of the device as compared with liquid feeding taking place from above the transport plane. By using this structure it is also easy to implement a large number of parallel process lines (in Figures 25a and 25b, three parallel process lines). For these reasons the option disclosed is advantageous in terms of productivity.

The option has the disadvantage that the application must be able to withstand carry-over between the tracks. Otherwise the entire track zone must at times be washed in a bulk manner or by circulating the washing liquid systematically in the risk areas.

In a three-dimensional architecture the track can also be implemented without the risk of chemical carry-over, i.e. without intersecting grooves, and by using sequential elements, directable in two directions (Figure 26a) and by using gradient electrodes, progressing in one direction but branched (Figure 26b). In this case a portion of the liquids is introduced from above, for example, with the help of piezos or manifolds. When the liquid is introduced onto the surface from above, the surface structure can be made simpler than when using feeding from the side. In this case the surface can be preferably made disposable.

The option depicted in Figures 26a and 26b does not contain intersecting travel tracks for different reagents but can be expanded further for a large number of parallel process lines.

Figure 27 depicts an option in which the surface of the device has been divided into a first surface 30 and a second surface 130. The first surface 30 is located at a higher level than the second surface 130. Thus, when it is desired to transfer droplets 51 from the first surface 30 to the second surface 130, the droplets are transported in the manner described above to the edge of the first surface 30, from where they drop onto the second surface 130 in accordance with Figure 27.

The double-level option depicted in Figure 27 has the advantage that thereby it is easy to divide the device into functionally different sections. For example, the second surface 130 can be made disposable, in which case, by using transfer means 131 it is possible, when so desired, to replace the second surface 130 without in any way affecting the first surface 30. It is not necessary to have on the second surface 130 the track network described above. The transfer of the droplet 51 can, when necessary, be effected by moving with the transfer means 131 the second surface 130, and thereby also the droplet thereon, in the desired direction.

Below, the method according to the invention is described with the help of examples. The detailed test arrangements and reaction conditions of the examples below are described in the publication "Sensitive Bioaffinity Assays on Individual Microparticles Using Time-Resolved Fluorometry," T. Lövgren et al., Clin. Chem. 43:10, 1997. The principle of the assay, used in Example 2, on the surface of an individual microparticle is depicted in Figures 28a and 28b.

In Figure 28a, there has been linked to the microparticle 241 a monoclonal antibody 242, to which the antigen 243 to be assayed has become attached. To the antigen 243 to be assayed there has further become attached a monoclonal antibody 244 labelled with label molecules 245.

In Figure 28b, there is linked to the microparticle 241 a synthetic oligonucleotide probe 246, to which the DNA 247 to be assayed becomes attached. A synthetic oligonucleotide probe 248 labelled with label molecules 245 becomes attached to the DNA 247.

The method according to the present invention is especially well suited for multiparameter analyses based on the use of microparticles, in which a mixture made up of microparticles of different types is used. Biospecific reagents binding different analytes are linked to the different types of microparticles. The different microparticle types can be distinguished one from another by means of type-specific labels. The microparticle mixture, the sample which contains a number of analytes to be assayed and labelled bioaffinity reagents specific for different analytes are incubated. Each microparticle gives two signals, one being characteristic of the microparticle type (i.e. analyte type) and the other detecting the analyte concentration. Multiparameter analyses such as this are described in the literature, for example in Finnish patent FI93781 and US patent 5,028,545.

The results according to Figures 25 and 26 are obtained by way of example from the assays described below (Examples 1-6) when there is used in the handling of the liquid a channel-free surface transport network for liquid droplets and the result is

measured from individual microparticles by using time-resolved fluorometry. In Example 1, reference is made to the reference numerals in Figures 23a, 23b, 24a and 24b.

Example 1. Demonstration of a prostate-specific antigen (PSA) by using a single-stage non-competitive immunoassay.

For the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined volume. The first capillary 231 doses the sample, the second capillary 232 doses an antibody-coated microparticle in a buffer solution, the third capillary 234 doses, in a buffer, an antibody labelled with a fluorescent europium chelate, and the fourth capillary 237 doses a washing solution.

The following work stages take place in the surface transport network:

A. The first capillary 231 doses the sample to the surface transport network.
B. The second capillary 232 doses an antibody-coated microparticle to the surface transport network.
C. The third capillary 234 doses an antibody labelled with a fluorescent europium chelate to the surface transport network.
D. The liquid droplets containing a sample droplet and a microparticle are fused within a first fusion zone 233, and the droplet thus obtained is fused with a droplet which contains a labelled antibody within a second fusion zone 235.
E. The fused droplet is transported on the track to a mixing point 236, where the contents of the droplet are mixed for a certain time. The PSA present in the sample

binds quantitatively and specifically to the antibody on the microparticle surface. Likewise the PSA binds a corresponding amount of the labelled antibody.

F. After the mixing stage the droplet is transported on the track to the washing point 238, where the washing of the microparticle takes place. The buffer and any excess antibody labelled with a fluorescent chelate are removed from around the microparticle.

G. The microparticle is washed with a washing solution, which comes to the washing point on the track from a fourth capillary 237.

H. If the detection point is at a point different from that of the washing, the microparticle is transported on the track in a washing solution droplet to the point where the labelled molecules on the surface of the microparticle are quantitated using fluorescence measurement. The quantity of fluorescent molecules is directly proportional to the quantity of PSA present in the sample. As was pointed out before, the measurement can also be carried out at the washing point 238, if the system has been so designed.

I. After the measurement, the droplet containing the microparticle is transported from the track to a waste container 239.

Example 2. Demonstration of prostate-specific antigen (PSA) by using a two-stage non-competitive immunoassay.

For the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined volume. Capillary I doses the sample, capillary II doses an antibody-coated microparticle in a buffer solution, capillary III doses, in a buffer, an antibody labelled with a

fluorescent europium chelate, and capillary IV doses a washing solution.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary II doses the antibody-coated microparticle to the surface transport network.

C. The sample droplet and the droplet which contains a microparticle are fused on the surface transport network.

D. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain period. The PSA present in the sample binds quantitatively and specifically to the antibody on the microparticle surface.

E. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. The buffer is removed from around the microparticle.

F. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

G. The microparticle is transported on the track in a washing solution droplet to a point where an antibody labelled with a fluorescent europium chelate is added to it; the antibody comes from capillary III.

H. The droplet which contains the microparticle is transported on the track to a point where the contents of the droplet are mixed for a certain time. The PSA on the

microparticle surface binds a corresponding amount of the labelled antibody.

I. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. Any excess antibody labelled with a fluorescent chelate is removed from around the microparticle.

J. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

K. The microparticle is transported on the track in a washing solution droplet to a point where the labelled molecules on the microparticle surface are quantitated using fluorescence measurement. The quantity of fluorescent molecules is directly proportional to the PSA quantity present in the sample.

L. After the measurement the droplet containing the microparticle is transported from the track to a waste container.

Example 3. Progesterone assay by using a competitive immunoassay.

In the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined volume. Capillary I doses the sample, capillary II doses, in a buffer solution, a microparticle coated with a progesterone-specific antibody, capillary III doses a progesterone derivative labelled with a fluorescent europium chelate, and capillary IV doses a washing solution.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary II doses a microparticle coated with a progesterone-specific antibody to the surface transport network.

C. Capillary III doses a progesterone derivative labelled with a fluorescent europium chelate to the surface transport network.

D. The droplets which contain the sample droplet, the microparticle and the labelled progesterone derivative are fused in the surface transport network.

E. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The progesterone present in the sample and the labelled progesterone derivative compete for binding to the progesterone-specific antibody on the microparticle surface.

F. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. The buffer and any excess progesterone derivative labelled with a fluorescent chelate are removed from around the microparticle.

G. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

H. The microparticle is transported on the track in a washing solution droplet to a point where the labelled molecules on the microparticle surface are quantitated using fluorescence measurement. The quantity of fluorescent molecules is inversely proportional to the quantity of progesterone present in the sample.

I. After the measurement, the droplet which contains the microparticle is transported from the track to the waste container.

Example 4. Demonstration of a gene defect which causes cystic fibrosis, by using a two-stage nucleic acid hybridization method.

In the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined size. Capillary I doses the sample, which contains, replicated, the gene fragment which contains a gene defect, capillary II doses, in a buffer solution, a microparticle coated with a synthetic nucleic acid probe, capillary III doses a nucleic acid probe labelled with a fluorescent europium chelate, and capillary IV doses a washing solution.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.
B. Capillary II doses a microparticle coated with a synthetic nucleic acid probe to the surface transport network.
C. The sample droplet and the microparticle-containing droplet are fused in the surface transport network.
D. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The DNA fragment in the sample, containing a gene defect, binds specifically to the synthetic nucleic acid probe on the microparticle surface.

E. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. The buffer and any remaining sample are removed from around the microparticle.

F. The microparticle is washed with a washing solution which comes to the washing point on the track from capillary IV.

G. The microparticle is transported on the track in a washing solution droplet to a point where a nucleic acid probe labelled with a fluorescent europium chelate is added to it; the probe comes to the track from capillary III.

H. The microparticle-containing droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. A portion of the nucleic acid probe labelled with a europium chelate binds to the DNA fragment which contains a gene defect, bound on the microparticle surface.

I. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. Any excess nucleic acid probe labelled with a fluorescent chelate is removed from around the microparticle.

J. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

K. The microparticle is transported on the track in a washing solution droplet to a point where the labelled molecules on the microparticle surface are quantitated using fluorescence measurement. The quantity of fluorescent molecules indicates directly whether the sample contains a gene defect which causes cystic fibrosis.

L. After the assay, the microparticle-containing droplet is transported from the track to a waste container.

Example 5. Demonstration of a genetic defect which causes cystic fibrosis, by using a single-stage nucleic acid hybridization method.

In the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined volume. Capillary I doses a sample which contains, replicated, a gene fragment which contains a gene defect, capillary II doses, in a buffer solution, a microparticle coated with a synthetic nucleic acid probe, capillary III doses a nucleic acid probe labelled with a fluorescent europium chelate, and capillary IV doses a washing solution.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary II doses a microparticle coated with a synthetic nucleic acid probe to the surface transport network.

C. Capillary III doses a nucleic acid probe labelled with a fluorescent europium chelate to the surface transport network.

D. The droplets containing the sample droplet, a microparticle and a labelled nucleic acid probe are fused in the surface transport network.

E. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The DNA fragment in the sample, containing a

gene defect, binds specifically to the synthetic nucleic acid probe on the microparticle surface and to the labelled nucleic acid probe.

F. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. Any excess of the labelled probe, the buffer and the remaining sample are removed from around the microparticle.

G. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

H. The microparticle is transported on the track in a washing solution droplet to a point where the labelled molecules on the microparticle surface are quantitated using fluorescence measurement. The quantity of fluorescent molecules indicates directly whether the sample contains a gene defect which causes cystic fibrosis.

I. After the measurement the microparticle-containing droplet is transported from the track to a waste container.

Example 6. Demonstration of a gene defect which causes cystic fibrosis, by replicating the target and by using a single-stage nucleic acid hybridization method.

For the assay there are required four separate capillary conduits, which are used for the dosing of liquid droplets of a predetermined volume. Capillary I doses a sample which contains a gene defect, capillary II doses the synthetic oligonucleotides (primers) required for the polymerase chain reaction (PCR, replication), one of the oligonucleotides being biotinylated, capillary III doses the nucleotides required for the replication reaction and polymerase (enzyme), capillary IV doses a streptavidin-coated microparticle in a buffer solution,

capillary V doses a nucleic acid probe labelled with a fluorescent europium chelate, and capillary VI doses a washing solution.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary II doses the primers to the surface transport network.

C. Capillary III doses the nucleotides required for the replication reaction and the polymerase (enzyme) to the surface transport network.

D. The droplets containing the sample droplet, the primers and the nucleotides, as well as the polymerase, are fused in the surface transport network.

E. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time and the temperature of the droplet on the track is regulated in the manner required by PCR replication.

F. The liquid droplet which contains the replicated target is transferred on the surface transport track to a point where a streptavidin-coated microparticle and a labelled oligonucleotide probe are added to it on the surface transport track from capillaries IV and V.

G. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The replicated DNA fragment containing a gene defect, present in the sample, the fragment containing a biotinylated primer, binds specifically to the

streptavidin on the microparticle surface and to the labelled nucleic acid probe.

H. After the mixing stage the droplet is transported on the track to a point where the washing of the microparticle takes place. Any excess of labelled probe, the buffer and the remaining sample are removed from around the microparticle.

I. The microparticle is washed with a washing solution, which comes to the washing point on the track from capillary IV.

J. The microparticle is transported on the track in a washing solution droplet to a point where the labelled molecules on the microparticle surface are quantitated using fluorescence measurement. The quantity of fluorescent molecules indicates directly whether the sample contains a gene defect which causes cystic fibrosis.

K. After the measurement the microparticle-containing droplet is transported from the track to a waste container.

For PCR replication it is possible to arrange on the track one or more points at which the temperature of the surface can be regulated with precision and speed in accordance with what has been stated before.

The detailed experimental arrangements and reaction conditions of the examples described below are described in reference 20, Hemmilä & al, "Homogeenisia määritysmenetelmiä, jotka perustuvat luminisenssienergiansiirtoon," ("Homogenous luminescence energy transfer assays") patent application No. FI-963989 and PCT/FI97/00560. The examples are not limiting; their purpose is to clarify the object of the invention in greater detail.

Example 7. Homogeneous energy transfer assay of β_{hCG}.

In the assay there are required two separate capillary conduits, which are used for dosing liquid droplets of a predetermined volume. Capillary I doses the sample, capillary II doses simultaneously both of the monoclonal antibodies used in the assay, one of them being labelled with Tb chelate (endopyrazole [20]) (donor) and the other being labelled with tetramethyl rhodamine (acceptor). These two antibodies bind to different epitopes in the β_{hCG} subunit and form a complex in which the energy transfer occurs. The fluorescence generated by the energy transfer by the acceptor bound to the complex is dependent on the quantity of β_{hCG} present in the sample. Figure 29 shows a typical calibration curve obtained in a homogeneous energy transfer assay for a β_{hCG} assay.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary II doses simultaneously both of the monoclonal antibodies used in the assay to the surface transport network.

C. The sample droplet and the droplet containing the labelled antibodies are fused in the surface transport network.

D. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The β_{hCG} present in the sample binds quantitatively and specifically to both of the labelled antibodies, which thus form a complex in which the energy transfer occurs.

E. After the mixing stage the droplet is transported on the track to a point where the tetramethyl rhodamine fluorescence of the formed complex is quantitated using fluorescence measurement. The quantity of fluorescent molecules is directly proportional to the quantity of β_{hCG} present in the sample.

F. After the measurement the droplet is transported from the track to a waste container.

Example 8. Homogeneous energy transfer assay of PSA (prostate-specific antigen).

In the assay there are required two separate capillary conduits for the dosing of liquid droplets of a predetermined volume. Capillary I doses the sample, capillary II doses simultaneously both of the monoclonal antibodies used in the assay, one of them being labelled with a Tb chelate (modified endopyrazole [20]) (donor) and the other being labelled with tetramethyl rhodamine (acceptor). These two antibodies bind to different epitopes of PSA and form a complex in which the energy transfer occurs. The fluorescence generated by the energy transfer by the acceptor bound to the complex is dependent on the quantity of PSA present in the sample. Figure 30 shows a typical calibration curve for the homogeneous energy transfer assay of PSA.

The following work stages take place in the surface transport network:

A. Capillary I doses the sample to the surface transport network.

B. Capillary H doses simultaneously both of the monoclonal antibodies used in the assay to the surface transport network.

C. The sample droplet and the droplet containing the labelled antibodies are fused in the surface transport network.

D. The fused droplet is transported on the track to a point where the contents of the droplet are mixed for a certain time. The PSA present in the sample binds quantitatively and specifically to both of the labelled antibodies, which thus form a complex in which the energy transfer occurs.

E. After the mixing stage the droplet is transported on the track to a point where the tetramethyl rhodamine fluorescence of the formed complex is quantitated using fluorescence measurement. The quantity of fluorescent molecules is directly proportional to the quantity of PSA present in the sample.

F. After the measurement the droplet is transported from the track to a waste container.

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Claims

1. A method for performing a chemical analysis, in particular a bioaffinity assay, on small liquid volumes, **characterized** in that droplets (51) are formed from the sample solution and from one of more solutions to be used in the assay, the droplets (51) being transported on a surface (30) repellant to them, by using an electric field of changing strength in order to fuse the droplets (51) in a desired order and optionally to carry out an analysis, to shake the droplet (51), to regulate the temperature, and/or to wash a solid carrier possibly contained in the droplet (51).

2. A method according to Claim 1, **characterized** in that the droplets (51) and the solid carrier, if any, are transported on the surface (30) in the desired direction by transferring the local electric field sequentially in the said direction.

3. A method according to Claim 1, **characterized** in that the droplets (51) and the solid carrier, if any, are transported on the surface (30) in the desired direction by means of an electric field gradient.

4. A method according to Claim 1, **characterized** in that the droplets (51) and the solid carrier, if any, are transported on the surface (30) in the desired direction by sequencing of an electric field gradient.

5. A method according to Claim 1, **characterized** in that the transport of the droplets (51) and the solid carriers, if any, on the surface (30) is promoted by causing the surface to vibrate mechanically.

6. A method according to Claim 1, **characterized** in that the continuous transport of the droplets (51) and the solid carriers, if any, on the surface (30) is promoted by means of the kinetic energy of the droplets.

7. A method according to Claim 1, **characterized** in that the droplets (51) are charged with static electricity by means of microscopic tips (91) in the surface.

8. A method according to Claim 1, **characterized** in that the droplets (51) are charged with static electricity by means of an ion source (101) outside the droplet.

9. A method according to Claim 1, **characterized** in that the droplets are transferred onto the surface (30) from a capillary tip (33) which has a static voltage.

10. A method according to Claim 1, **characterized** in that the droplets are transferred onto the surface (30) from a capillary tip (33) which has a dynamic voltage.

11. A method according to Claim 1, **characterized** in that droplets (51) of an electrically conductive liquid are transferred onto the surface (30) from a capillary tip (33) and are charged electrically.

12. A method according to Claim 1, **characterized** in that the droplet (51) is touched with a charged tip (101) in order to change the charge level of the droplet (51).

13. A method according to any of the above claims, **characterized** in that the droplet (51) is mixed by moving the droplet reciprocally.

14. A method according to any of the above claims, **characterized** in that the droplets (51) are heated, when so desired, locally by means of heating elements (181; 191; 192; 193) [in the surface].

15. A method according to Claim 14, **characterized** in that the droplets (51) are heated locally by using resistive heating elements (181).

16. A method according to Claim 14, **characterized** in that the droplets (51) are heated locally by using a high frequency electric field.

17. A method according to any of Claims 14-16, **characterized** in that the temperature of the heating points is changed between two or more temperatures.

18. A method according to any of Claims 14-16, **characterized** in that there are two or more heating points (201, 202, 203, 204; 205, 206; 207, 208, 209) in such a manner that the heating points are at preset temperatures and the temperature of the reaction mixture is changed by moving a droplet (51) from one heating point to another and back, according to need, in order to produce the desired temperature sequence.

19. A method according to any of the above claims, wherein the reaction product is immobilized on an individual solid carrier in a droplet (51), **characterized** in that any unbound

reagent is removed before the measurement by removing the solution surrounding the solid carrier through an opening (171) narrower than the solid carrier and, when necessary, by replacing it with a droplet of washing liquid.

20. A method according to any of the above claims, wherein the reaction product is immobilized on an individual solid carrier in a droplet (51), **characterized** in that the droplet containing the solid carrier is stretched, by means of the said electric field, beyond its breaking point, whereafter a droplet (167) of washing solution is fused with the droplet portion containing the solid carrier.

21. A method according to any of the above claims, **characterized** in that there is performed a two-stage non-competitive immunoassay, a single-stage non-competitive immunoassay, or a competitive immunoassay.

22. A method according to any of the above claims, **characterized** in that there is performed a single- or two-stage nucleic acid hybridization assay.

23. A method according to any of the above claims, **characterized** in that a time-resolved fluorescence label is used in the assay.

24. A device for the performing of a chemical analysis on small liquid volumes, having means for transporting and fusing (52) liquid doses, and optionally for dosing (33) a liquid volume containing a sample and at least one liquid volume containing a reagent and, if a solid carrier is used, a washing liquid volume, for shaking (92, 93) the reaction mixture, for

regulating (181; 191; 192, 193) the temperature, and/or for analyzing the reaction product, **characterized** by a liquid-repellent surface (30), electrodes (52) fitted as transport tracks in the vicinity of the surface (30), and means (33) for transferring liquid volumes as droplets to the surface in the area of the said transport tracks.

25. A device according to Claim 24, **characterized** in that at least some of the electrodes (52) are beneath the liquid-repellent surface (30).

26. A device according to Claim 24, **characterized** in that at least some of the electrodes (52) are above the liquid-repellent surface.

27. A device according to Claim 24, **characterized** in that at least some of the electrodes (52) are on the sides of the droplet (51) travel route.

28. A device according to Claim 24, **characterized** in that the electrodes (51) are in a paired array in a position wedged in the orientation of the transport track in order to provide an electric field which strengthens sequentially in the orientation of the transport track.

29. A device according to Claim 28, **characterized** in that at least in a portion of the transport track there are at least two paired arrays (92, 93) of electrodes in wedged positions in opposite directions for the changing of the droplet (51) travel direction.

30. A device according to Claim 24 or 28, **characterized** in that there are electrodes (52) along the length of the transport track, the electrodes (52) being capable of being sequentially coupled to a voltage source (81) in order to produce an electric field which progresses in the orientation of the transport track.

31. A device according to Claim 24, **characterized** in that there is coupled to the transport track a piezoelectric crystal (111) for producing vibration in the surface (30).

32. A device according to Claim 30 or 31, **characterized** in that the transport track surface common to the droplet (164) containing a solid carrier and the droplet (167) consisting of washing liquid has a narrowing passage which is narrower than the solid carrier (165), the solid carrier (165) being washed therein.

33. A device according to Claim 30 or 31, **characterized** in that the surface of the transport track for the droplet (164) which contains a solid carrier has a narrowing passage, narrower than the solid carrier (165), in which the solid carrier (165) can be parked precisely for measurement.

34. A device according to Claim 24, 28, 29, 30 or 31, **characterized** in that the surface of the transport track common to the droplet which contains a solid carrier and the droplet consisting of washing liquid has an aperture (171) which passes through the surface and is narrower than the solid carrier (165), the aperture communicating with means for sucking the liquid from around the solid carrier (165) above of the aperture.

35. A device according to any of Claims 24-34, **characterized** by a field emitter (91, 101) in the surface (30) or above it, in the area of the transport track, for charging the droplets (51) and for regulating their transport properties.

36. A device according to any of Claims 24-35, **characterized** in that the material of the surface (30) is in the area of the transport tracks less liquid-repellent and/or less rough than in the area of the rest of the surface.

37. A device according to any of Claims 24-36, **characterized** by a casing enveloping the surface (30) and by means for regulating the moisture and temperature of the atmosphere inside the casing.

38. A device according to any of Claims 24-37, **characterized** in that the device has means (61) for isolating the droplet (51) in a space of small volume at least at one point of the transport track.

39. A device according to Claim 38, **characterized** in that the said means (61) comprise at least an elastic casing fitting over the droplet (51).

40. A device according to any of Claims 24-39, **characterized** in that, when so desired, the surface (30) can be heated by using the transport electrodes (52, 191, 192; 193).

41. A device according to any of Claims 24-40, **characterized** in that, when so desired, the droplet (51) is heated by high-frequency electricity.

42. A device according to any of Claims 24-41, **characterized** in that the surface (30) is a planar substrate.

43. A device according to any of Claims 24-41, **characterized** in that at least a portion of the surface (30) is disposable.

44. A device according to any of Claims 24-43, **characterized** in that the device has a second, preferably disposable surface (130), which is in a plane different from that of the first surface (30) in such a manner that the droplets (51) leaving the first surface (30) drop onto the second surface (130).

45. A device according to Claim 44, **characterized** in that at least the second surface (130) is disposable.

46. A device according to Claim 44, **characterized** in that the device has means (131) for moving the second surface (130).

47. A device according to Claim 44, **characterized** in that the second surface (130) is at least in part a surface permeable to the liquid forming the droplet (51).

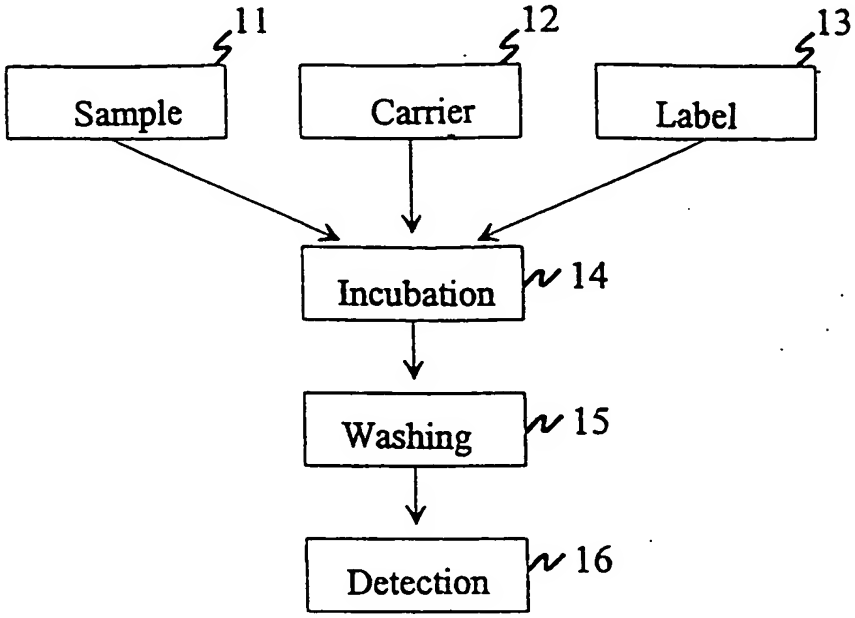


Fig. 1

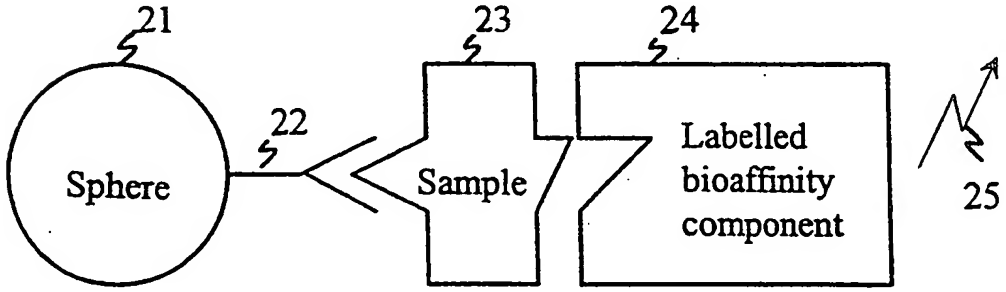


Fig. 2

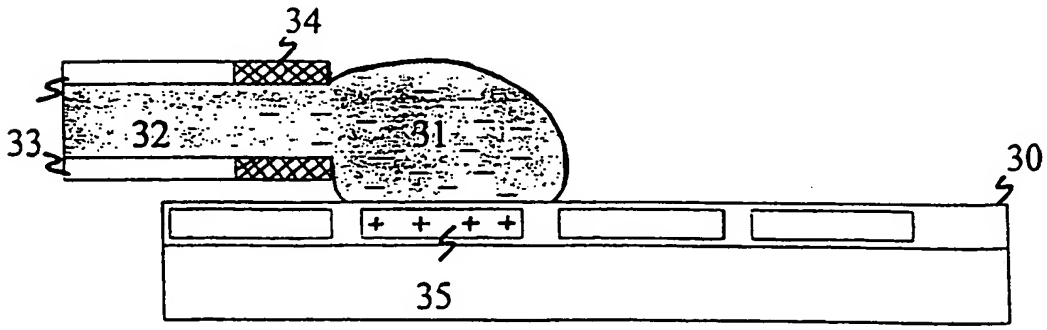


Fig. 3

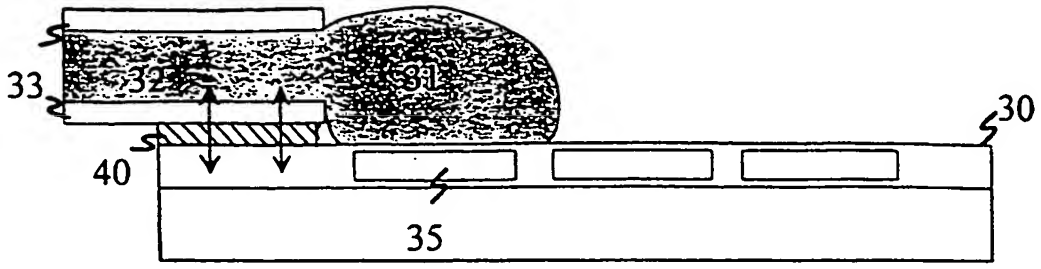
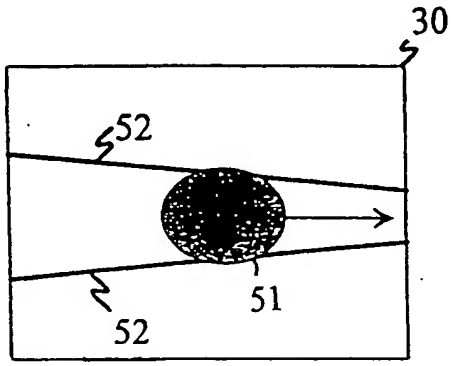
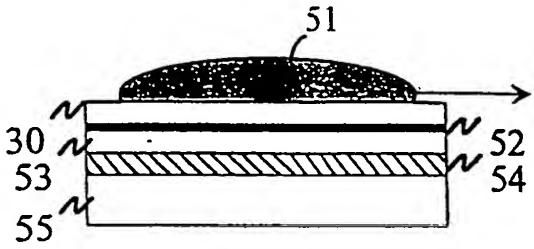


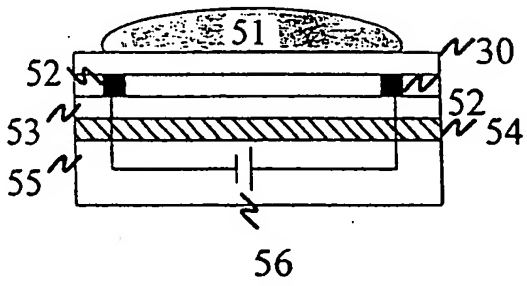
Fig. 4



From above
Fig. 5a



From the side
Fig. 5b



From the front/behind
Fig. 5c

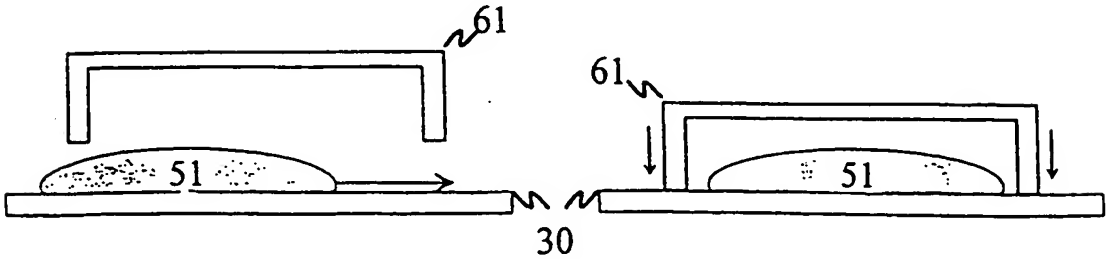
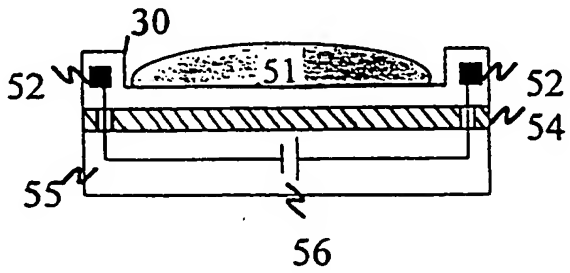
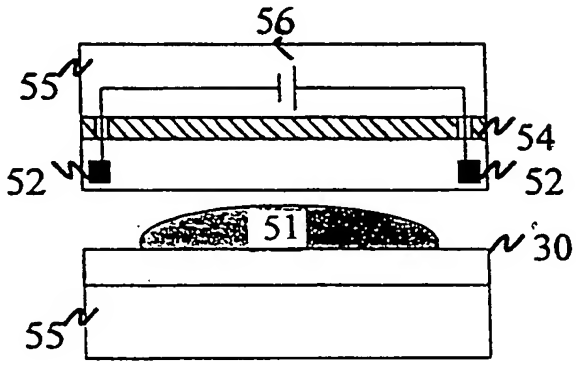


Fig. 6



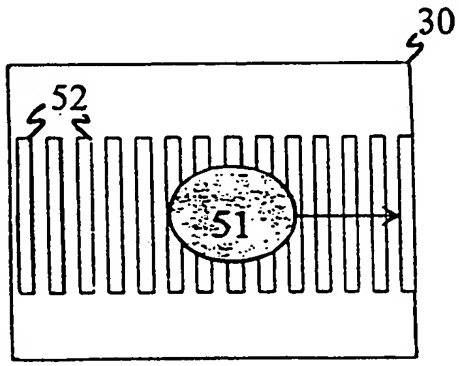
From the front/behind

Fig. 7



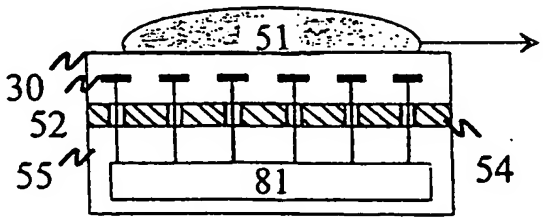
From front/behind

Fig. 8



From above

Fig. 9a



From the side

Fig. 9b

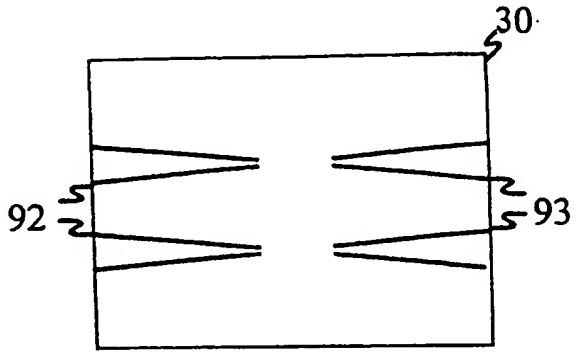


Fig. 10a

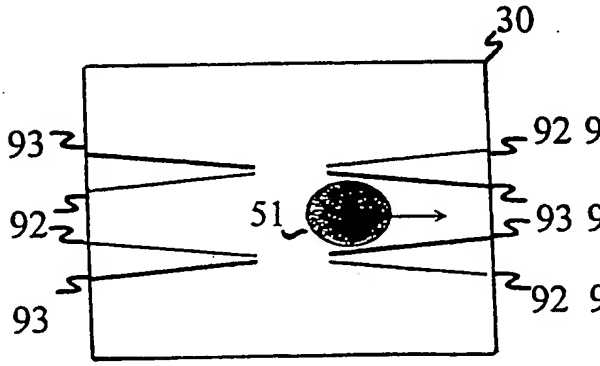


Fig. 10b

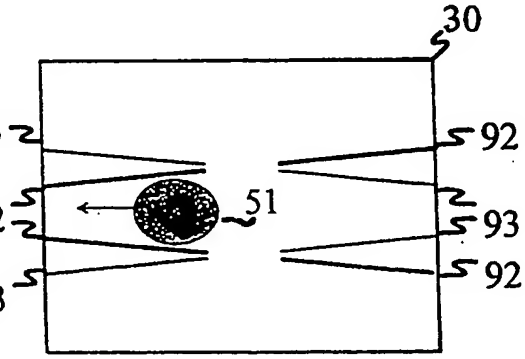


Fig. 10c

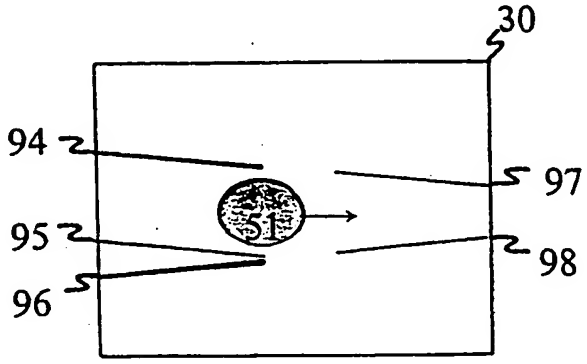


Fig. 11a

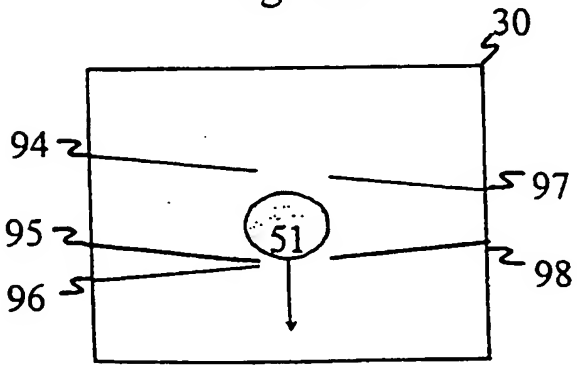


Fig. 11b

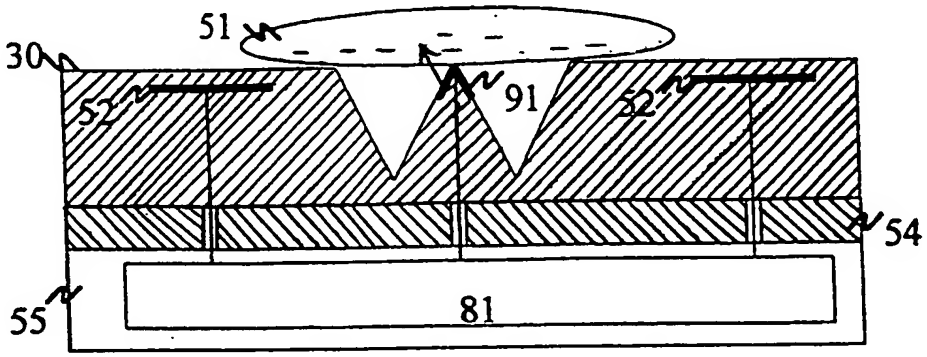


Fig. 12

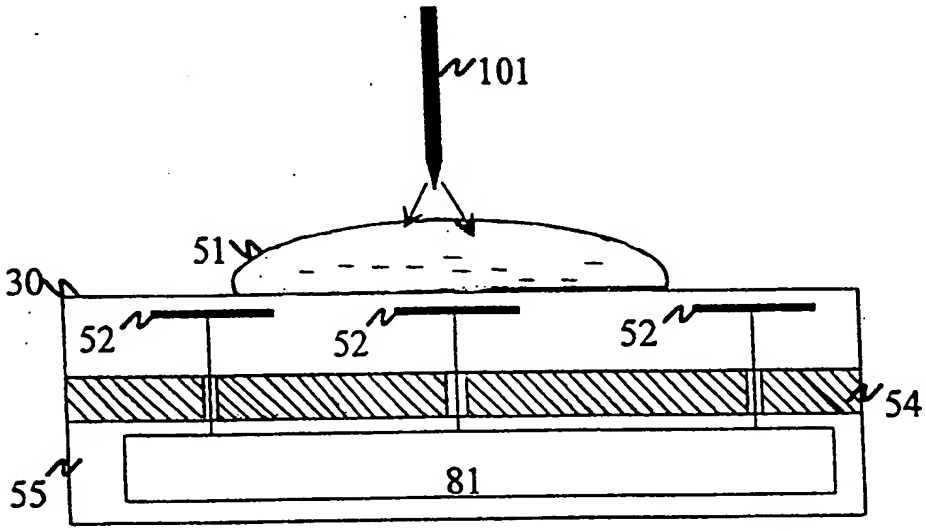


Fig. 13

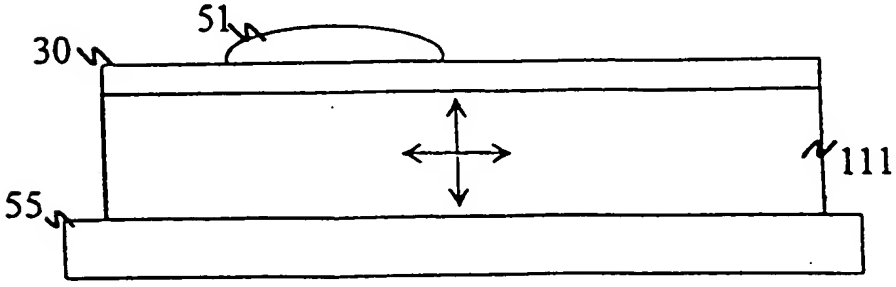


Fig. 14

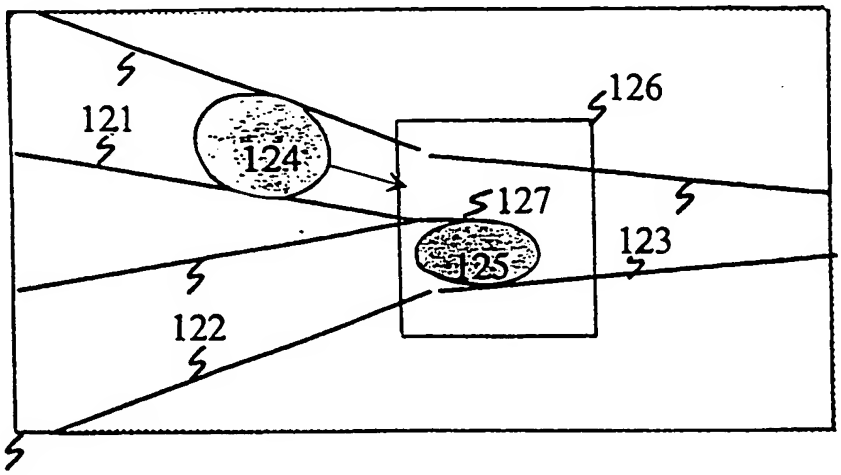


Fig. 15

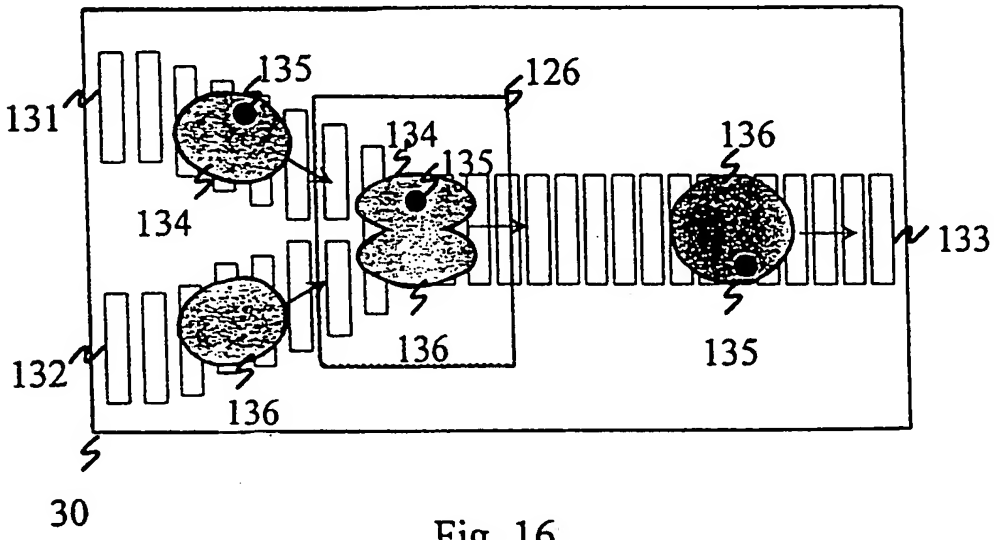


Fig. 16

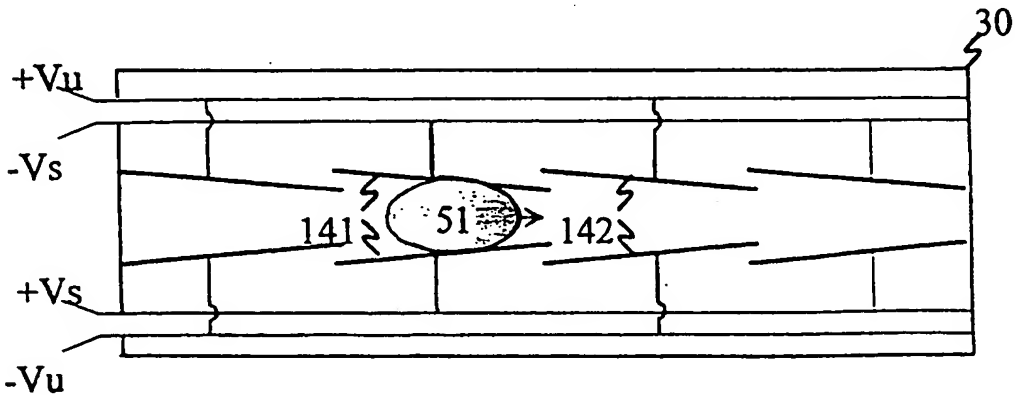


Fig. 17

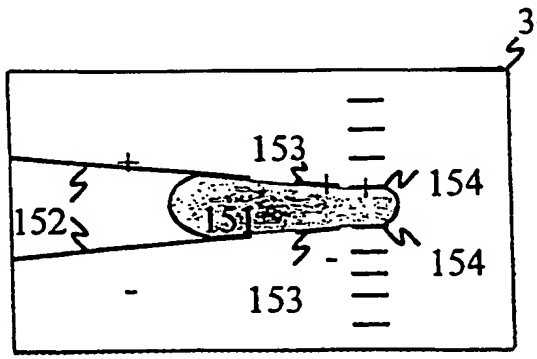


Fig. 18a

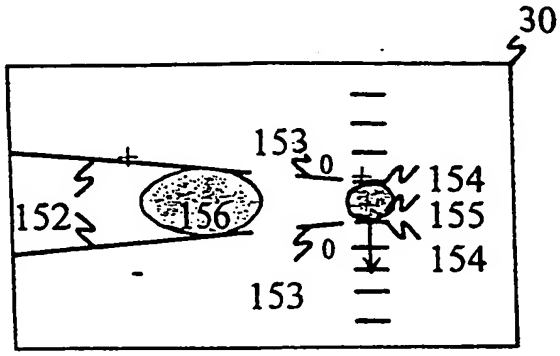


Fig. 18b

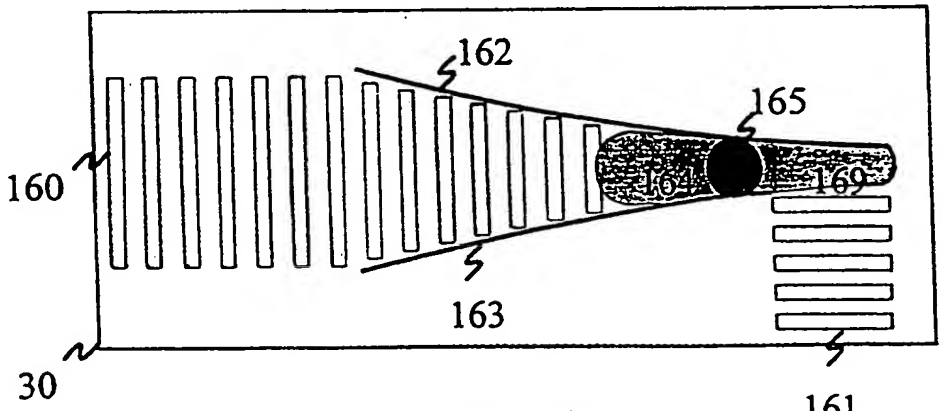


Fig. 19a

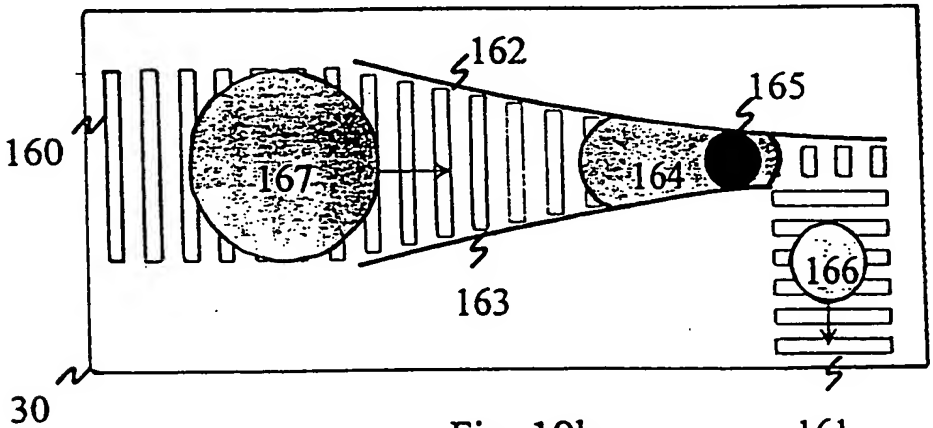


Fig. 19b

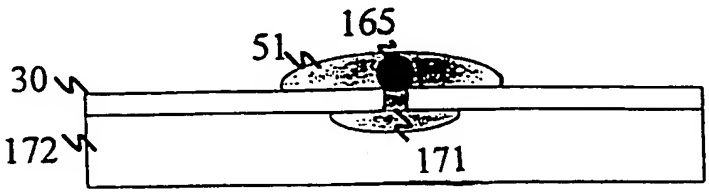


Fig. 20

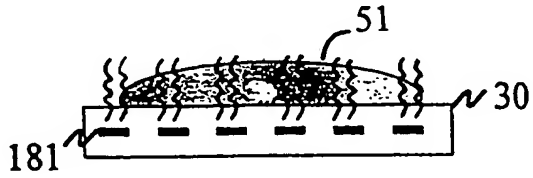


Fig. 21

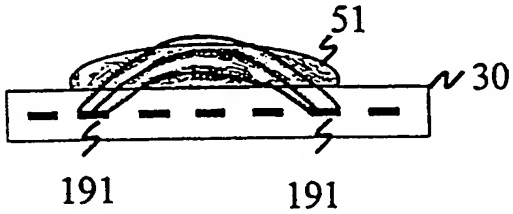


Fig. 22a

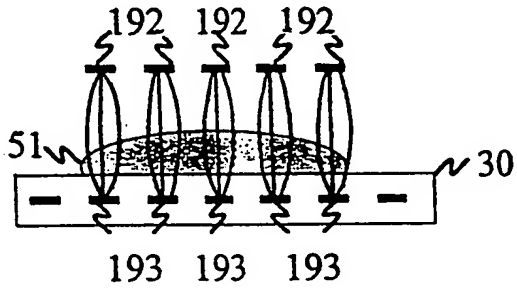


Fig. 22b

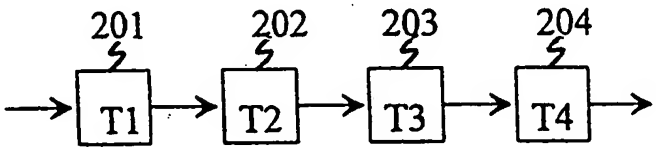


Fig. 23a

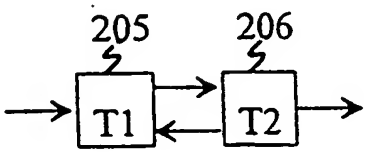


Fig. 23b

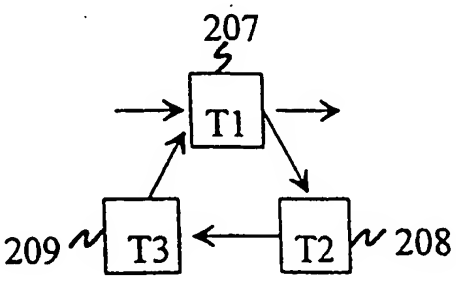


Fig. 23c

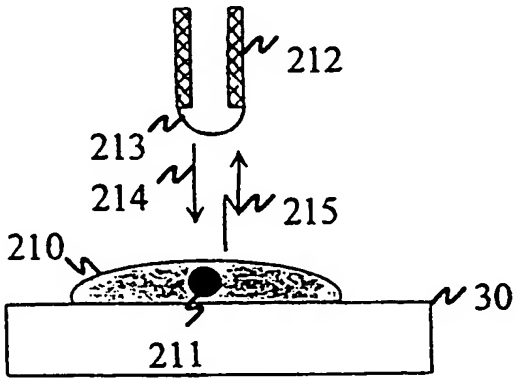


Fig. 24

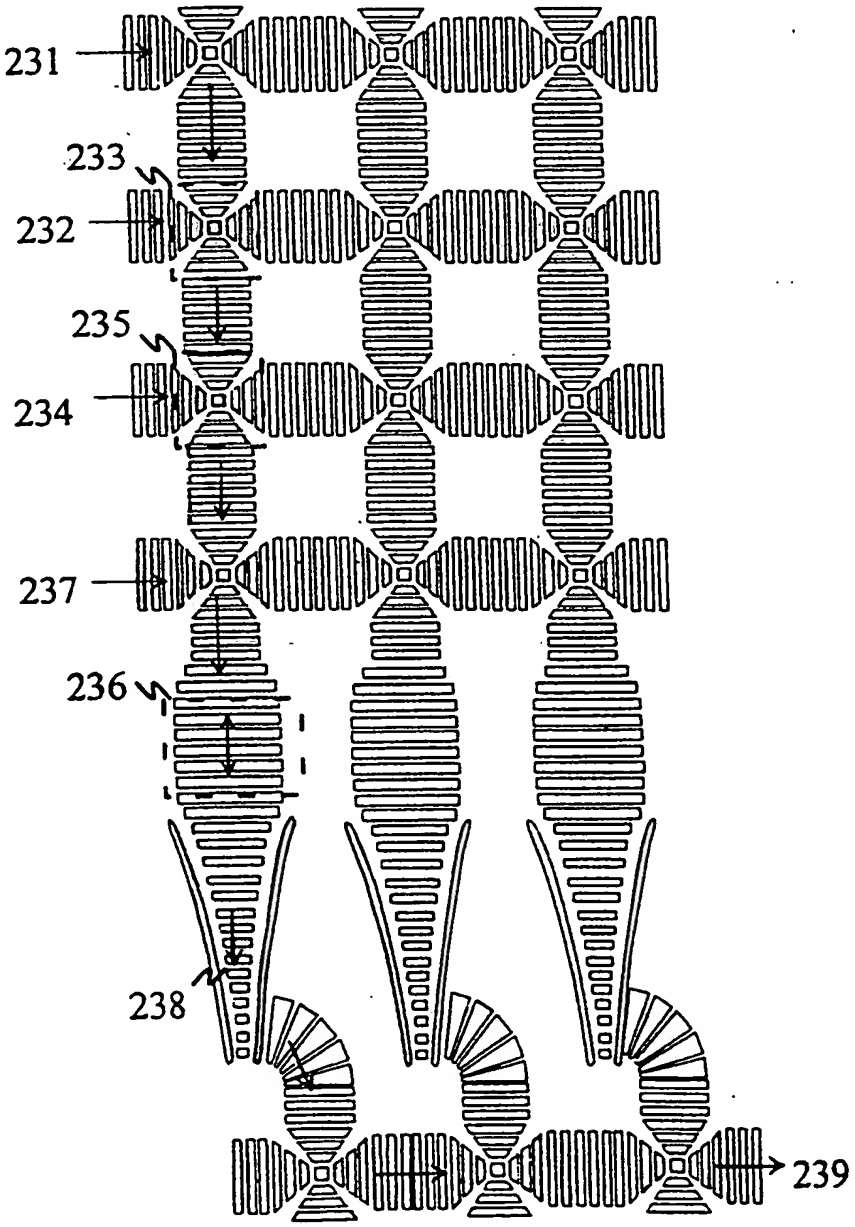


Fig. 25a

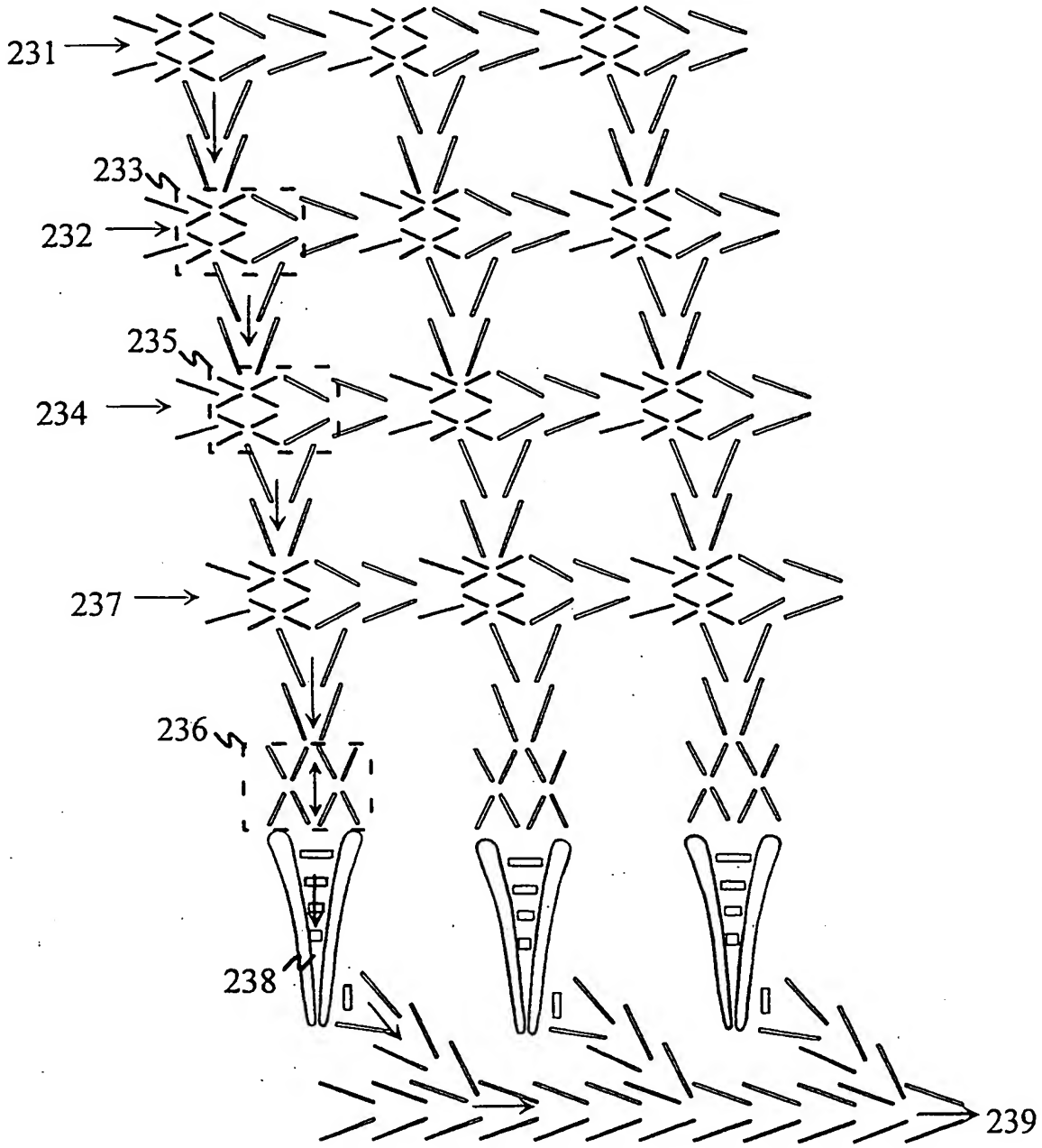


Fig. 25b

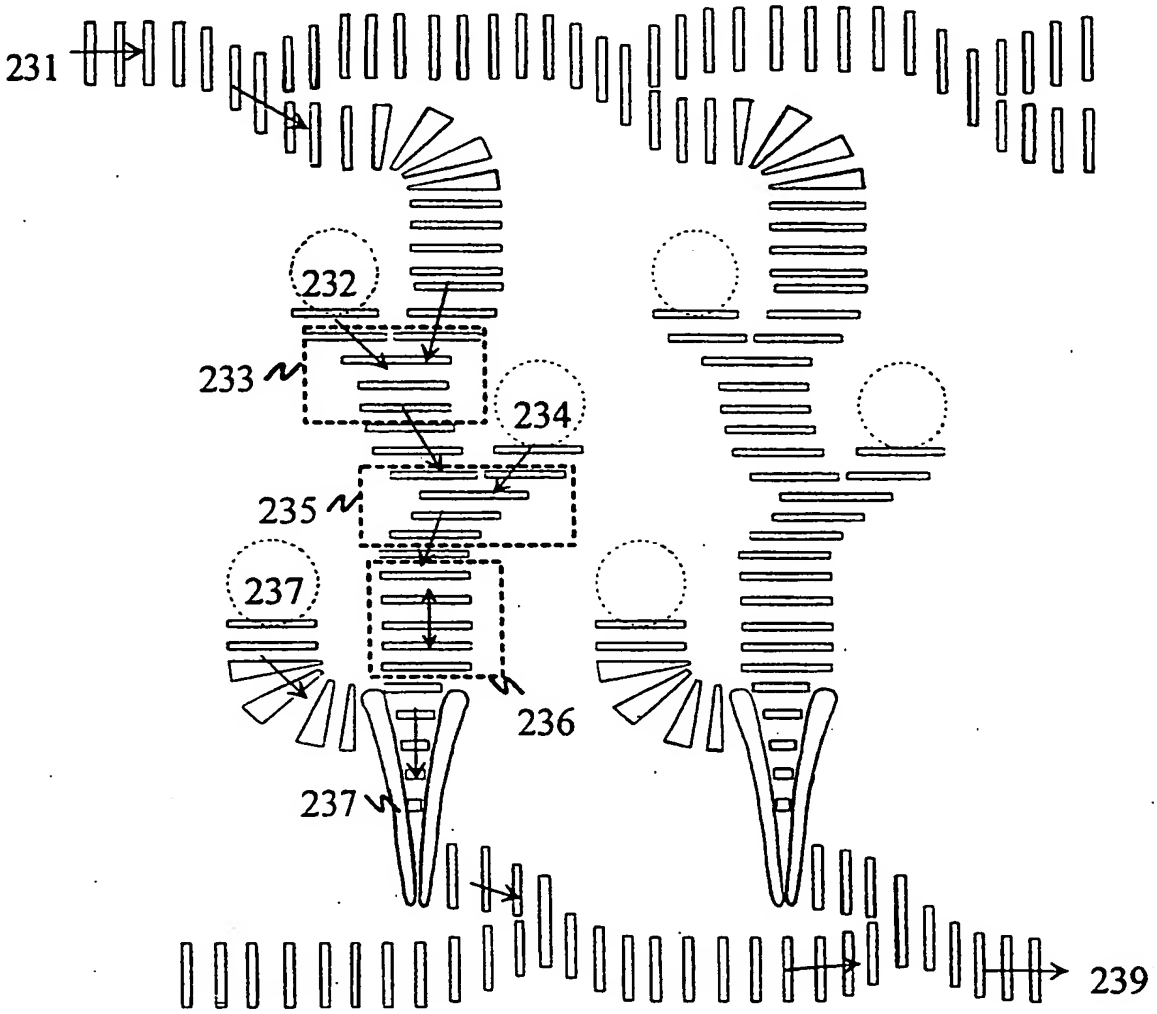


Fig. 26a

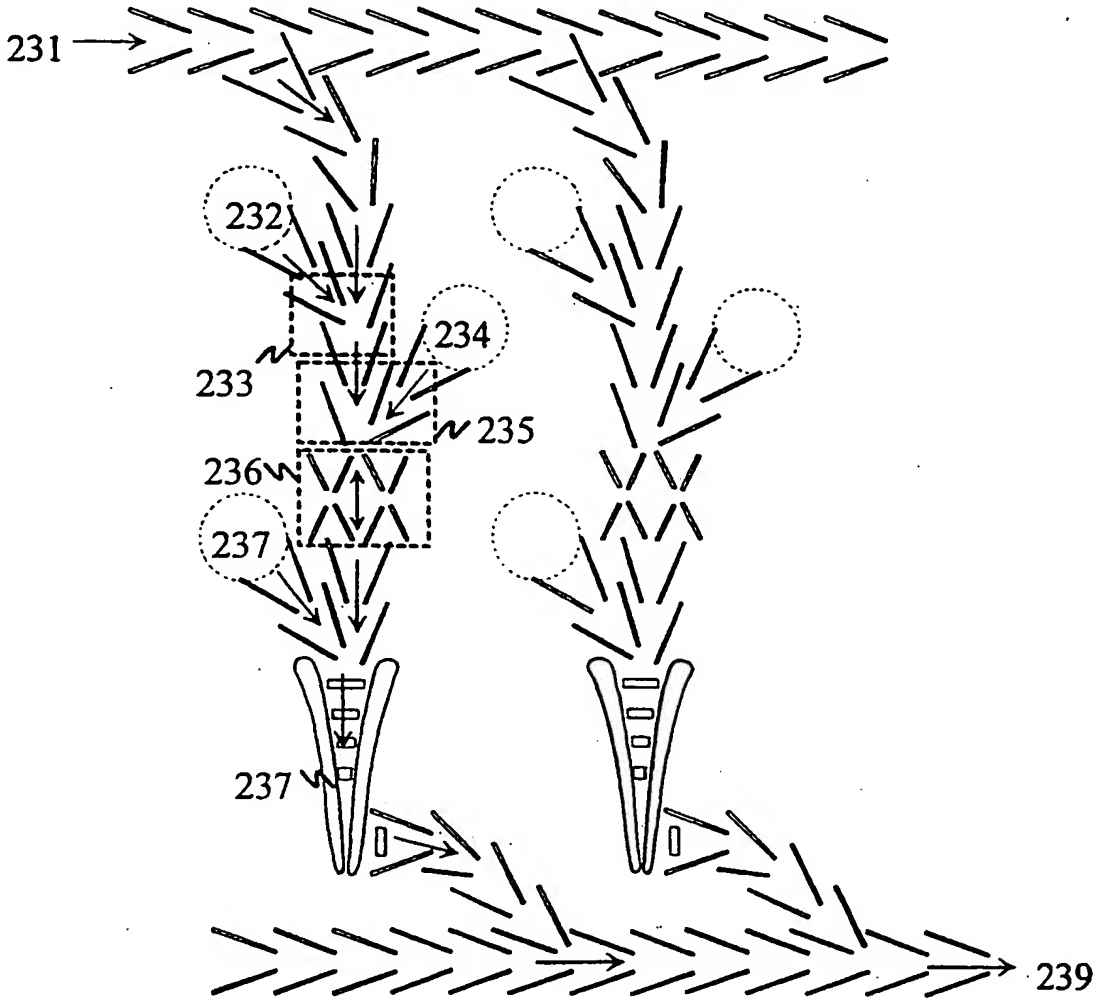


Fig. 26b

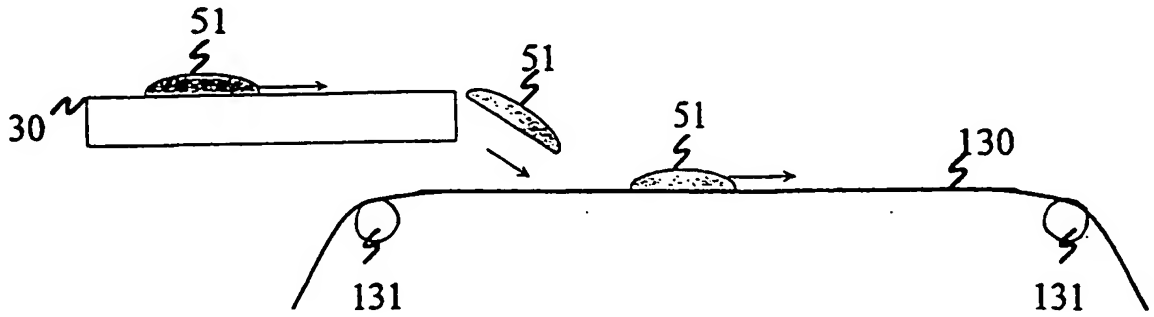


Fig. 27

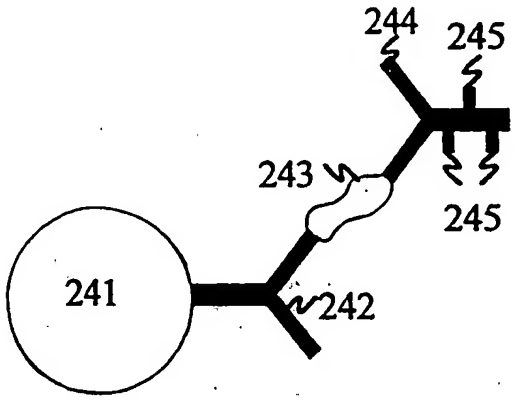


Fig. 28a

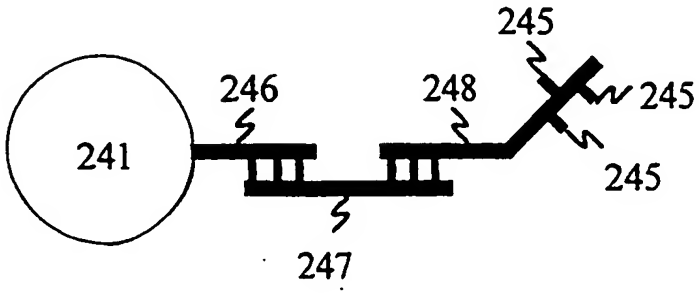


Fig. 28b

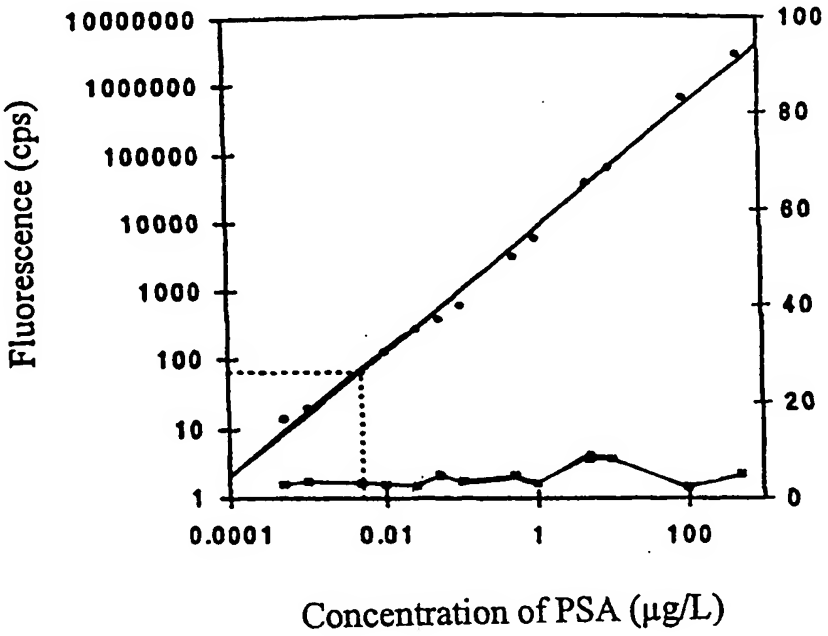

10000000
1000000
100000
10000
1000
100
10
1
100
80
60
40
20
0
0.0001
0.01
1
100
Fluorescence (cps)
Concentration of PSA (µg/L)

Fig. 29

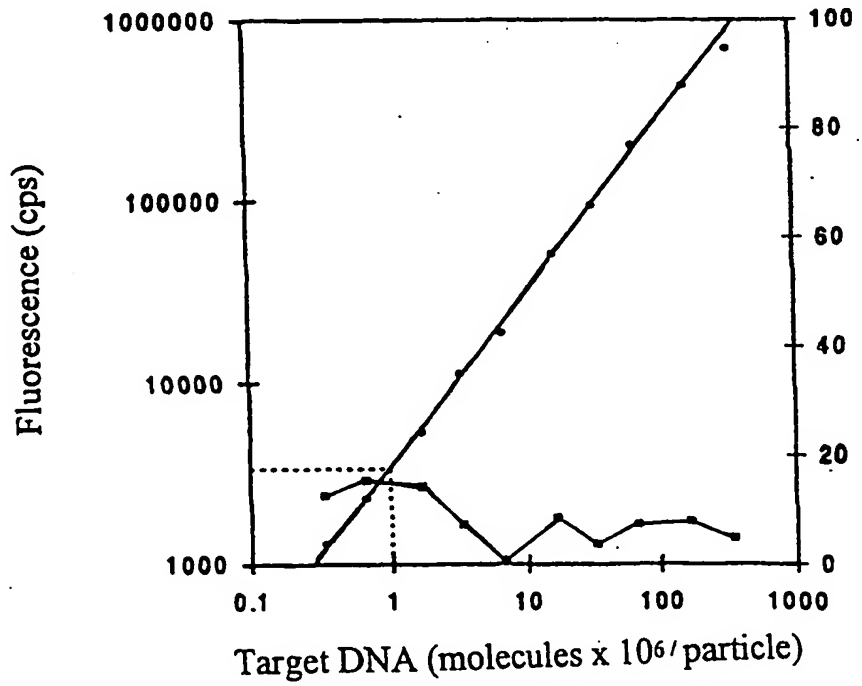

1000000
100000
10000
1000
100
80
60
40
20
0
0.1
1
10
100
1000
Fluorescence (cps)
Target DNA (molecules x 10⁶ / particle)

Fig. 30

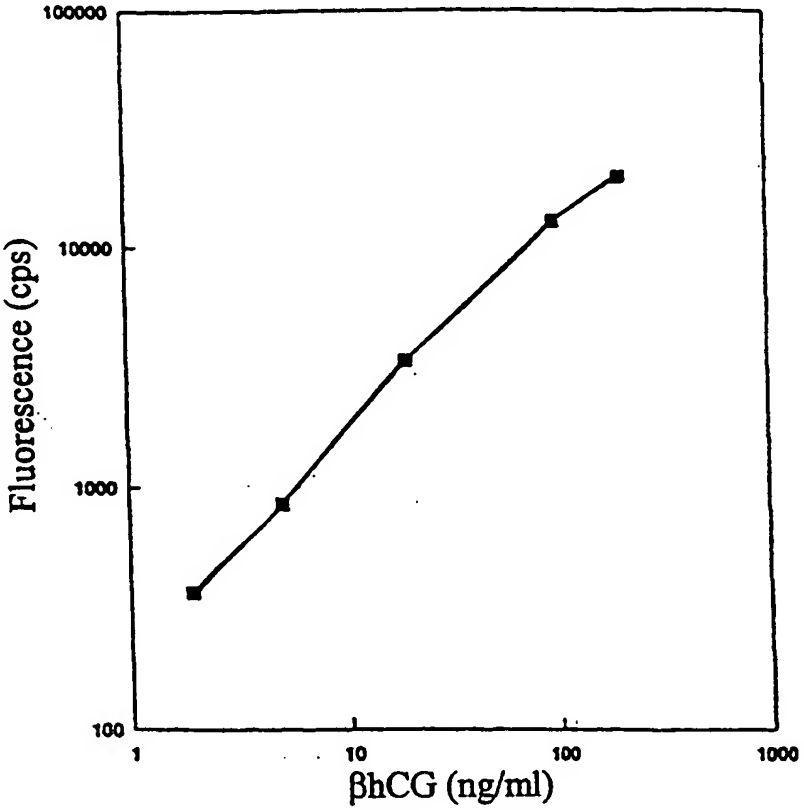

100000
10000
1000
100
Fluorescence (cps)
1
10
100
1000
βhCG (ng/ml)

Fig. 31

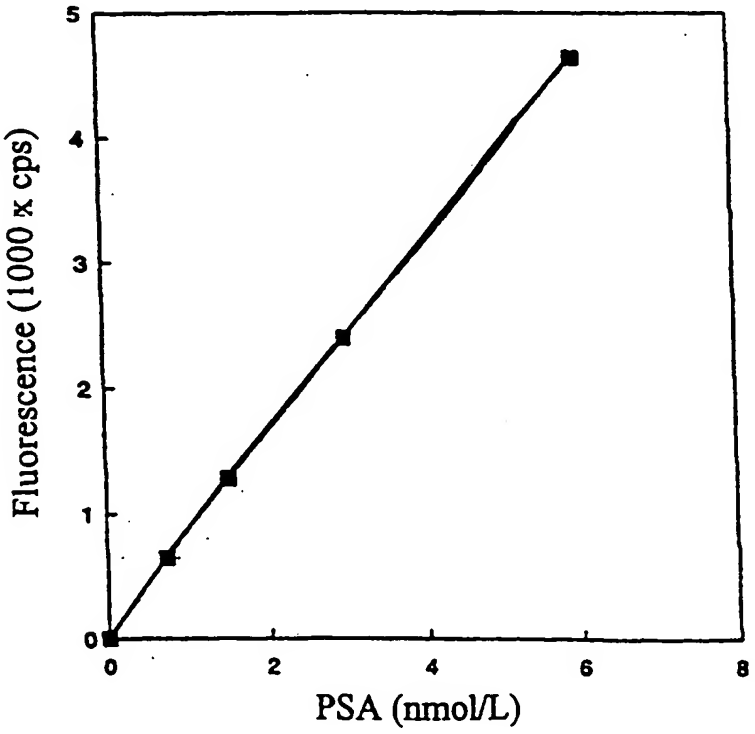

5
4
3
2
1
0
Fluorescence (1000 x cps)
0
2
4
6
8
PSA (nmol/L)

Fig. 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00309

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/53, G01N 1/28, G01N 1/38, B01F 5/00, B01F 11/02
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N, H02N, B01F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, PATENT ABSTRACTS OF JAPAN, MEDLINE, BIOSIS, SCI-SEARCH, CASEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IEEE Transactions on industry applications, Volume 34, No 4, 1998, Masao Washizu, "Electrostatic Actuation of Liquid Droplets for Microreactor Applications", page 732 - page 737, see entire document, presented at the Industrial Applications Society Annual Meeting, New Orleans, LA, October 5-9, 1997	1-4,6,13-16, 24-30,37, 41-43
Y	--	17,21-23
X	US 5486337 A (TIHIRO OHKAWA), 23 January 1996 (23.01.96), see entire document	1-4,6-13, 24-30,35-36, 42-43
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 1999	04-08-1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/EÖ Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00309

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	New Scientist, 1 March 1997, Mark Ward, "Devilish tricks with tiny chips", pages 22-26 --	17,21-23
A	Dialog Information Service, File 155, MEDLINE, Dialog accession no. 09289597, Medline accession no. 98000063, Lovgren T et al: "Sensitive bioaffinityassays with individual microparticles and time-resolved fluorometry"; Clin Chem (UNITED STATES) Oct 1997, 43 (10) p1937-43, see abstract --	23

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/07/99

International application No.
PCT/FI 99/00309

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5486337 A	23/01/96	NONE	